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# PROTEIN PRODUCTION FROM ACID WHEY VIA FERMENTATION

Sheldon Bernstein, et al

Milbrew, Incorporated

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16. Abstract

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Economic estimates show that while a large capital investment and low cost raw material are required for the commercial feasibility of this fermentation process, it will be competitive with other methods for the manufacture of single cell protein. This whey fermentation is one means of converting large quantities of a potential environmental pollutant into a useful and needed product. (EPA)

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# PROTEIN PRODUCTION FROM ACID WHEY VIA FERMENTATION

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## ABSTRACT

From the operation of a demonstration pilot plant over extended periods of time, it has been shown that yeast may be grown on an acid whey or sweet whey medium in a continuous, deep tank aerated fermentor. Variations in fermentation conditions, strain selection, and medium composition produced cell concentrations of several billion cells per milliliter. By a process of evaporation and spray drying the whole fermented whey mass and the utilization of the evaporator condensate to dilute incoming condensed whey, a high grade, non-toxic, protein feed material may be produced without any effluent streams. Amino acid analyses and protein efficiency ratios are presented for this feed material.

Economic estimates show that while a large capital investment and low cost raw material are required for the commercial feasibility of this fermentation process, it will be competitive with other methods for the manufacture of single cell protein. This whey fermentation is one means of converting large quantities of a potential environmental pollutant into a useful and needed product.

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## SECTION I

## SUMMARY AND CONCLUSIONS

### INTERNATIONAL MINERALS CORPORATION-PRELIMINARY STUDIES

1. A series of experiments were conducted in 44 liter, pilot scale fermentors using several strains of lactose-fermenting Saccharomyces. The process of conversion of lactose in whey to yeast cellular material was reasonably efficient with conversion rates of 45-55% recorded. Therefore, the literature conversion rate of 55% was attained (11).
2. The fermentation process was completed in 8-10 hours and could be operated either in a semi-continuous manner with minimum "downtime" or continuous manner without "downtime".
3. Cell yields, cell counts, and crude protein concentrations were approximately equivalent for the various fermentation processes.
4. From a fermentation standpoint the process is simple to operate and maintain (non-sterile) and should become an effective means of converting whey to a marketable commodity.

## SECTION II

## CONCLUSIONS AND RECOMMENDATIONS

## AMBER LABORATORIES

1. Saccharomyces fragilis can be produced at growth levels of several billion cells per milliliter using a deep tank aerated fermentor operated in a continuous manner on acid or sweet whey.

2. The complete whole fermented whey mass may be dried eliminating effluents and produce a satisfactory feed supplement. The yield of fermented whey mass (FWM) was 0.675 to 0.75 pounds per pound of whey solids.

3. A superior quality yeast, high in protein and low in ash content, may be produced by harvesting the yeast cells. The product should find commercial applications in specialty feeds and in foods as a food additive.

4. The fermentation could be carried out on permeates from ultrafiltration (UF) of acid or sweet whey. However, numerous problems were encountered using the UF unit such as low flux rates due to membrane fouling, inability to properly sanitize the UF unit, and extremely low solids in the lactose permeate. Therefore, the fermentation of lactose permeate from the UF system studied does not look economically feasible.

5. A large capital investment and low cost, raw material are required for the commercial feasibility of the fermentation process. These studies were limited to the laboratory and pilot development of protein production from acid and sweet

whey via yeast fermentation. It is recommended that the scaled-up testing of protein production from whey fermentation in commercial sized fermentors be examined which would permit full-scale, long term evaluation of the chemical, physical, biological and economic aspects of the process.

## SECTION III

## INTRODUCTION

### INTERNATIONAL MINERALS CORPORATION

The preliminary investigation and shake flask studies were done by International Minerals Corporation, Libertyville, Illinois. Various Standard Operating Procedures were developed and used by Amber Laboratories in the operation of the 500 gallon, Demonstration Pilot Plant fermentor. The results of the Amber Laboratories studies are presented in Sections VI-IX.

The carbon of whey lactose can be converted by a variety of lactose-fermenting yeasts to yeast cellular components of which 50% are protein. An exogenous source of inorganic nitrogen is usually required as a supplement since whey protein is not a readily available N-source for these saccharolytic strains. It has been considered that if fermentation processes were devised which could result in near theoretical conversion of lactose to yeast protein in a suitably short fermentation cycle then such a process might prove a feasible means of producing a low cost protein feed supplement from the high tonnage of whey which is currently considered waste material.

As discussed in a preliminary report (International Minerals Corporation, Section XIII Appendices) shake flask experiments demonstrated that sufficient doublings of yeast populations in short fermentation cycles could be obtained on simple whey

media to warrant further investigation of the process in pilot fermentors (15 gallon). Plant throughputs of 2-3X fermentor capacity per 24 hour period appeared feasible. The shake flask experiments also demonstrated that such a process might be run under non-sterile conditions.

The following series of experiments was carried out in fermentors as an extension of this work. A progression from batch to semi-continuous to continuous fermentor operation was carried out to demonstrate the optimum method for use of fermentor capacity and feasibility of bhe whey to yeast conversion process.

## SECTION IV

## MATERIALS AND METHODS

### INTERNATIONAL MINERALS CORPORATION

Two yeast strains, Saccharomyces fragilis (NURL, Y-1109) and S. lactis (NURL, Y-1140), were used throughout the fermentor experiments. The strains were maintained on a weekly transfer schedule on sterile whey agar.

Whey concentrate as received contained 35-40% total solids and a lactose content of 35-38%. The high lactose concentrate was prepared from locally collected raw acid whey by Amber Laboratories at Juneau, Wisconsin, and transported hot to the IMC fermentation pilot plant. Whey for the experiments described later was diluted to appropriate levels of lactose with tap water and then used tc prepare Medium 5102-A or Medium 5102-B. Prior to dilution, whey concentrate was heated to just below boiling to insure complete solubilization of lactose.

Whey agar was prepared by diluting whey concentrate with tap water to 5% $\pm$ 0.5% reducing sugar content and adding the components of 5102-A Medium described later. The pH was adjusted to 7.0 with 10% NaOH and agar added to a 2% concentration. The medium was heated to melt the agar, tubed in suitable aliquots and sterilized for 10 minutes at 120°C.

Two basal media were used throughout these studies. Medium 5102-A consisted of whey concentrate that was adjusted to 6% lactose concentration. The diluted concentrate was made to

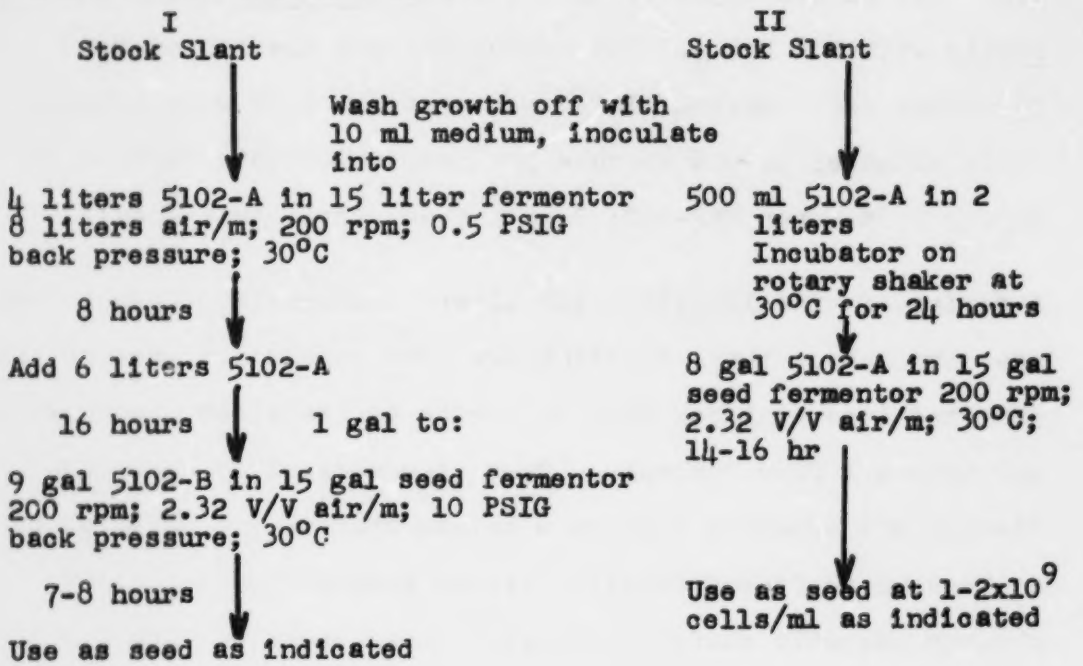
contain (W/V) 0.5% $(NH_4)_2SO_4$; 0.5% $K_2HPO_4$ and 0.1% Amber BYF-300. The pH was adjusted to 4.5 for S. fragilis and 5.5 for S. lactis with 10% $H_3PO_4$. The medium was not sterilized for fermentor use. Medium 5102-B was prepared from whey concentrate adjusted to ~6% lactose but made to contain (W/V) 0.9% $NH_4OH$; 0.3% Amber BYF-300; 0.001% FD-82 antifoam (Hodag).

A series of nine 15-gallon (44 liter) fermentation vessels was used throughout these experiments. The fermentors were of the jacketed type equipped with automatic pH, antifoam, temperature and nutrient feed controls. They were aerated by compressed sterile air entering through a bottom sparger and agitated by a standard shaft and impeller system powered hydraulically through magnetic shaft couplings. Steam could be directly injected into the vessel or circulated through the jacket. Cooling water could also be circulated through the jacket. The vessels were fitted with various inoculating and sampling ports and could be fitted to handle oxygen or carbon dioxide sensors. The series of fermentors were inter-connected by header lines allowing complete flexibility of material transfer from vessel to vessel. The series of 15-gallon fermentors was served by three 15-liter New Brunswick fermentors which served as seed vessels. The New Brunswick fermentors were of standard internal baffle design and were connected to the 15-gallon fermentor header line.

Two procedures were used for inoculum and are designated appropriately in the experimental summary (Table 1). The

procedures are outlined below:

Inoculum Buildup Procedures:


I
Stock Slant
Wash growth off with 10 ml medium, inoculate into
4 liters 5102-A in 15 liter fermentor
8 liters air/m; 200 rpm; 0.5 PSIG back pressure; 30°C
8 hours
Add 6 liters 5102-A
16 hours
1 gal to:
9 gal 5102-B in 15 gal seed fermentor
200 rpm; 2.32 V/V air/m; 10 PSIG back pressure; 30°C
7-8 hours
Use as seed as indicated
II
Stock Slant
500 ml 5102-A in 2 liters
Incubator on rotary shaker at 30°C for 24 hours
8 gal 5102-A in 15 gal seed fermentor 200 rpm; 2.32 V/V air/m; 30°C; 14-16 hr
Use as seed at 1-2x10^9 cells/ml as indicated

Fifteen gallon fermentors were inoculated from either of the trains indicated above as noted for individual experiments. The 10% level was standard. Fermentor operating conditions unless otherwise noted were 200 rpm; 2.32 V/V air/min; 10 PSIG back pressure; 30°C. The fermentors were not sterilized nor was pH controlled. Fermentors were loaded with 9 gal of medium and inoculum transferred from a seed vessel to bring the total fermentor volume to 10 gal (10% inoculum).

A three stage continuous fermentation procedure in the 15 gallon fermentors utilized an inoculum development as in I above. Three fermentors were simultaneously loaded and

inoculated. After six hours of inoculum development the fermentors were loaded to 9 gallon volume with 5102-B medium and a continuous feed of 5102-B medium to the first stage initiated. The rate of feed was calculated to accomplish total utilization of lactose by completion of the third stage. The rate of continuous feed was ≅3.18 gal/hr (14.0 liters/hr).

Solids determinations were made as described below: A sample representing approximately 1 gram of dried solids was weighed into a previously dried, cooled, and weighed 5 cm circular aluminum flat-bottomed dish containing reagent grade seasand (20/40 mesh). The dish and contents were heated at 60°C overnight at full vacuum (~30"). The dish was cooled in a dessicator and then reweighed. Protein was determined in accord with the A.O. A.C. method (11th Ed. 1970 #16.035 ref 2.051). A factor of 6.38 x percent-N was used as recommended for dairy product protein. Fat was determined in accord with the A.O.A.C. method (11th Ed. 1970 #16.052) for dairy products. Ash was determined in accord with the A.O.A.C. method (11th Ed. 1970 #16.034) for dairy products. Free and total reducing sugar was determined using a Technicon Autoanalyzer. The Technicon Ferricyanide colorimetric method was used to determine reducing sugars before and after hydrolysis. Total amino acids were determined by over-night hydrolysis in 6N HCl followed by column chromatography in a Beckman 120C Amino Acid Analyzer. Cell count analyses on samples of appropriate dilutions of whey fermentor broth were made in 0.1 M phosphate buffer, pH

7.0. Both chambers of a hemocytometer (Spencer Bright Line Improved Neubauer 1/10 mm deep) were loaded with the same diluted sample and only the large subdivided center square was used in counting.

If cell numbers were $<10^8$/ml the entire square was counted, the microscopic counts from both chambers were averaged and cells/ml calculated. If the two counts deviated from each other, the hemocytometer was reloaded, two more counts taken and the four counts averaged. In counting samples with high cell numbers, three rows of five squares each were counted for both chambers, the six counts averaged and cells/ml calculated in accord with the following equation:

$$\text{cells/ml} = \frac{\text{count} \times \text{dilution} \times 4 \times 10^3}{\#\ \text{small squares counted}} \times 10^3$$

Cell volumes were determined by centrifuging a 10 ml broth sample in a conical, graduated centrifuge tube at 250 rpm for 30 minutes in an International PR-2 centrifuge equipped with a fixed rotor. Volume of the pellet was read, in ml, directly from the tube.

## SECTION V

## EXPERIMENTAL RESULTS

### INTERNATIONAL MINERALS CORPORATION

The series of experiments described below were performed to determine the effects of inoculum size (Expt's 4,5), medium ingredients (Expt's 6-14), carbohydrate level (Expt's 12-17) and physical conditions (Expt's 16-17) on the two strains of yeast under study. In certain of the experiments operation of the fermentation under semi-continuous (Expt's 11,12,15) or continuous (Expt 18) conditions was examined. Experimental conditions and parameters are summarized in Table 1. The data obtained for maximum cell yield, total solids, dry weight of protein (as is), dry weight of washed protein, % conversion and % alcohol (ethanol) are provided where such determinations were made on a particular run.

The effect of amount of inoculum transferred to the fermentor on cell growth and yield were determined in Expts 4 and 5 for S. fragilis and S. lactis, respectively. Growth of S. fragilis under different inoculum size conditions is summarized graphically in Figure 1. The experiments were conducted under conditions in which carbohydrate level was continuously maintained at 5-6%. The data obtained were interpreted as reflecting the inhibitory effect of excess whey on growth of the yeasts which had been observed previously in shake flasks. The effect was not grossly apparent during the first four hours of fermentation. Doubling times for the yeasts calculated for this time

Table 1. SUMMARY OF EXPERIMENTAL DATA

| Condition | Medium | Max. Pop. cells/ml x10$^9$ | % Dry Weight T.S. "as is" | % Dry Weight Protein "as is" | % Dry Weight Protein washed | % Conversion | % Alcohol |
|---|---|---|---|---|---|---|---|
| **Experiment #4. Effect of inoculum size on S. fragilis fermentation** | | | | | | | |
| | 5-6% CHO W-10 | | | | | | |
| 44% | Inoculum | (4)* | 1.155 | 13.05 | 18.03 | 41.99 | |
| 25% | ↓ | (10) | 1.07 | 11.30 | 19.08 | | |
| 15% | | (10) | 1.07 | 10.40 | 19.84 | | |
| 5% | | (10) | .885 | 8.9 | 20.42 | | |
| 10% | | (10) | .795 | 8.8 | 22.22 | 43.60 | |
| 10% | Whey only | (8) | .805 | 8.2 | 15.51 | | |
| **Experiment #5. As in 4 S. lactis** | | | | | | | |
| | W-10 | | | | | | |
| 44% | Inoculum | (10) | 8.75 | 12 7 | | | |
| 25% | ↓ | (10) | 6 5 | 10.5 | | | |
| 15% | | (10) | 9.5 | 9.5 | | | |
| 5% | | (10) | 3.2 | 9.1 | | | |
| 10% | | (10) | 8.4 | 8.1 | | | |
| 2% | Whey only | (10) | 3.3 | 8.9 | | | |

*Numbers in parentheses refer to hours of fermentation at which maximum cell population was reached.

Table 1. (cont.)

**Experiment #6.** Effect of level of BYF and ammonium salts on *S. fragilis* fermentation

| BYF | $(NH_4)_2SO_4$ | W-10 | 10% In | | | | |
|---|---|---|---|---|---|---|---|
| 0.1% | 1.0% | (10) | 1.31 | 6.15 | | | 23.74 |
| 0.3 | 1.0 | (10) | 1.78 | 6.45 | 36.42 | 43.58 | 29.15 |
| 0.5 | 1.0 | ( 8) | 1.40 | 6.55 | | | 28.34 |
| 0.1 | 0.5 | ( 8) | 1.56 | 5.51 | 27.95 | 42.49 | 30.88 |
| 0.3 | 0.5 | ( 8) | 1.30 | 6.00 | | | 27.33 |
| 0.5 | 0.5 | ( 8) | 1.03 | 6.25 | 28.13 | | 36.23 |

**Experiment #7.** As in 6 *S. lactis*

| BYF | $(NH_4)_2SO_4$ | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.1% | 1.0% | (10) | 2.25 | 6.15 | 33.44 | 45.36 | 42.75 |
| 0.3 | 1.0 | (10) | 1.97 | 6.55 | 32.88 | 45.74 | 45.38 |
| 0.5 | 1.0 | (10) | 1.97 | 5.30 | 35.00 | 44.78 | 46.25 |
| 0.1 | 0.5 | (10) | 2.37 | 5.20 | 26.44 | 46.93 | 46.84 |
| 0.33 | 0.5 | (10) | 2.32 | 5.40 | 26.62 | 48.23 | 37.77 |
| 0.5 | 0.5 | (10) | 2.10 | 5.40 | 28.00 | 46.45 | 39.26 |

**Experiment #8.** Effect of N-source and P-level on *S. fragilis* whey fermentation

| BYF | $NH_4OH$ | $KH_2PO_4$ | NS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.3% | - | 0.5% | 0.5% | (10) | 2.91 | 6.15 | 26.25 | 45.74 | 36.83 |
| ↓ | - | 0.7 | 0.5 | (10) | 1.97 | 6.00 | 27.50 | | |
| ↓ | - | 1.0 | 0.5 | (10) | 2.35 | 6.00 | 26.25 | | |
| ↓ | - | 0.5 | 1.0 | (10) | 1.90 | 5.35 | 38.31 | | |
| ↓ | 0.45% | 0.5 | - | (10) | 1.64 | 5.05 | 27.81 | 37.51 | 61.03 |
| ↓ | 0.90 | 0.5 | - | ( 8) | (1.57) | 6.00 | 34.50 | 41.09 | 56.15 |

Table 1. (cont.)

**Experiment #9.** As in 8 *S. lactis*

| N-OH | $KH_2PO_4$ | NS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| - | .5% | 0.5% | (10) | 3.15 | 5.40 | 29.67 | 41.09 | 53.72 |
| - | .7 | 0.5 | (10) | 3.11 | 5.95 | 25.01 | | |
| - | 1.0 | 0.5 | (10) | 3.08 | 6.20 | 27.88 | 36.11 | 55.66 |
| - | 0.5 | 1.0 | (10) | 3.26 | 5.55 | 36.43 | | |
| 0.45% | 0.5 | - | ( 8) | (2.17) | 5.10 | 28.97 | 37.13 | 54.36 |
| 0.90 | 0.5 | - | ( 8) | (2.39) | 5.80 | 34.32 | | |

**Experiment #11.** Effect of $NH_4OH/H_3PO_4$ medium on *S. fragilis* fermentation

| | $NH_4OH$ adj. | $H_3PO_4$ | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.9% | | ( 8) | 1.10 | - | - | - | |
| 1A | | | (10 | 1.08 | | | | |
| 1B | | | ( 9) | 1.22 | | | | |
| 2 | | | (10) | 1.12 | 6.80 | 31.58 | 33.30 | 50.09 |
| 2A | | | (10) | 1.31 | | | | |
| 2B | | | ( 9) | 1.08 | | | | |
| 3 | 0.45 | | ( 8) | 1.23 | 6.65 | 25.47 | 33.56 | 55.5 |
| 3A | | | (10) | 1.08 | | | | |
| 3B | | | ( 9) | 1.29 | | | | |
| 4 | | | ( 8) | 1.05 | 6.40 | 24.88 | 33.56 | 52.42 |
| 4A | | | (10) | 1.01 | | | | |
| 4B | | | ( 9) | 1.22 | | | | |
| 5 | 0.9 | | (10) | 1.03 | 7.40 | 28.96 | 36.17 | 52.11 |
| 5A | | | (10) | 1.10 | | | | |
| 5B | | | ( 9) | 1.20 | | | | |

Table 1. (cont.)

**Experiment #12.** As in 11 *S. lactis*

| | $NH_4OH$ | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | 0.9% | (9½) | 3.09 | 6.95 | 31.33 | 35.91 | 49.05 |
| 1A | | (9) | 3.65 | | | | |
| 1B | | (9) | 3.30 | | | | |
| 2 | | (9) | 3.01 | 6.50 | 31.96 | 34.46 | 48.72 |
| 2A | | (9) | 3.40 | | | | |
| 2B | ↓ | (9) | 3.00 | | | | |
| 3 | 0.45 | (9) | 2.57 | 6.30 | 29.92 | 39.08 | 40.10 |
| 3A | | (9) | 3.10 | | | | |
| 3B | | (9) | 3.00 | | | | |
| 4 | | (9) | 2.98 | 7.05 | 30.11 | 39.08 | 40.24 |
| 4A | | (9) | 2.75 | | | | |
| 4B | ↓ | (9) | 2.80 | | | | |
| 5 | 0.9 | (9) | 3.00 | 7.00 | 33.30 | 37.50 | 45.07 |
| 5A | | (9) | 3.20 | | | | |
| 5B | ↓ | (9) | 3.00 | | | | |

**Experiment #13.** Effect of $K^+$ source and lactose level on *S. fragilis* fermentation

| $KH_2PO_4$ | $NH_4OH$ | Lactose | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.5% | 0.9% | 5-6 | (10) | 1.49 | 7.90 | 29.54 | 34.83 | 37.07 | 2.05 FWC |
| - | 0.45 | 5-6 | (10) | 1.32 | 6.25 | 32.98 | 40.38 | 28.10 | 2.33 |
| - | 0.90 | 5-6 | (10) | 1.29 | 7.15 | 32.98 | 38.73 | 33.60 | 2.63 |
| - | 0.90 | 10-11 | (14) | 1.23 | 11.75 | 26.16 | 31.64 | 26.80 | 1.75 |
| - | 0.45 | 10-11 | (14) | 1.23 | 10.40 | 25.01 | 36.24 | 21.34 | 0.78 |
| - | 0.90 | 10-11 | (14) | 1.25 | 10.60 | 27.88 | 34.13 | 24.30 | 1.05 |

Table 1. (cont.)

**Experiment #14.** As in 13 *S. lactis*

| $KH_2PO_4$ | $NH_4OH$ | Lactose | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.5% | 1.0% | 5 | (10) | 2.85 | 6.76 | 35.80 | 35.91 | 39.45 | 1.25 |
| ↓ | 0.5 | ↓ | (10) | 2.88 | 5.66 | 36.04 | 29.50 | 27.83 | 2.55 |
| | 1.0 | | (10) | 3.34 | 6.86 | 34.40 | 25.93 | 29.74 | 0.68 |
| | 1.0 | 10 | (12) | 4.00 | 10.43 | 31.16 | 36.53 | 35.56 | 0.33 |
| | 0.5 | ↓ | (14) | 4.70 | 10.15 | 26.40 | 37.47 | 24.12 | 0.53 |
| ↓ | 1.0 | ↓ | (12) | 3.30 | 10.43 | 31.16 | 36.56 | 27.91 | 0.20 |

**Experiment #15.** Effect of lactose level on semi-continuous operation of *S. fragilis* fermentation

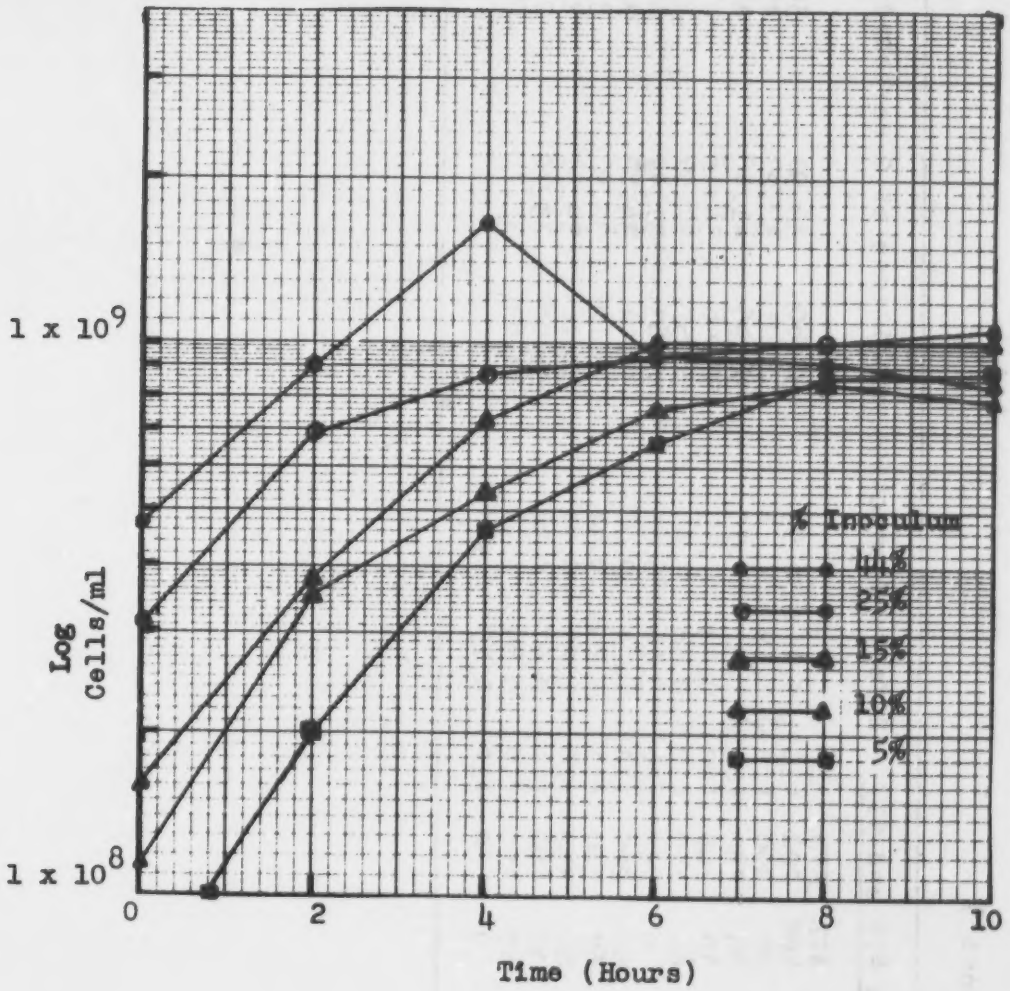
| | | | Lactose | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | (W-1) | | 5-6 | (8¾) | 0.98 | | | | 1.42 |
| 1A | (W-3) | Sub-1 | ↓ | ( 8) | 1.11 | | | | 1.53 |
| 1B | (W-5) | Sub-2 | ↓ | ( 8) | 0.92 | 5.48 | 46.53 | 40.00 | 56.04 | 1.76 |
| 2 | (W-2) | | 10-11 | (12) | 1.26 | | | | 2.86 |
| 2A | (W-4) | Sub-1 | | (10) | 1.15 | 9.87 | 37.48 | 34.14 | 54.00 | 3.38 |
| 2B | | Sub-2 | | (11) | 1.37 | 9.12 | 39.91 | 33.16 | 50.63 | 4.40 |
| 2C | (W-1 | Sub-3 | | (10) | 1.45 | | | | | 3.93 |
| 2D | (W-2) | Sub-4 | | (9½) | 1.40 | | | | | 4.30 |
| 2E | (W-3) | Sub-5 | | (11) | 1.44 | | | | | 4.85 |
| 2F | (W-4) | Sub-6 | ↓ | (12) | 1.40 | | | | | (0.65) |

Table 1. (cont )

Experiments #16 and #17. Effect of aeration conditions at 2 carbohydrate levels

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (16) | CHO | AIR | RPM | | | | | | | |
| | 5-6% | 50% | 200 | (10) | 4.1 | 6.93 | 37.80 | 35.91 | 36.25 | 2.28 |
| | | 60 | 300 | (10¾) | 1.19 | 7.51 | 38.21 | 37.27 | 42.06 | 1.76 |
| | | 80 | 350 | (10¾) | 1.31 | 7.70 | 38.96 | 41.48 | 41.56 | 1.36 |
| | | 30 | 100 | (10) | 0.73 | 7.15 | 37.48 | 34.16 | 29.72 | 3.26 |
| | | 40 | 150 | (10) | 0.88 | 7.44 | 38.57 | 37.07 | 26.63 | 3.26 |
| | | 0 | 50 | (10) | 0.16 | 6.61 | 39.63 | 31.47 | 13.6 | 3.65 |
| (17) | 10-11 | 50 | 200 | (13) | 1.24 | 9.08 | 33.04 | 34.83 | 30.2 | 4.02 |
| | | 60 | 300 | (11) | 1.70 | 9.34 | 32.12 | 32.9 | 36.02 | 3.26 |
| | | 80 | 350 | (10) | 1.23 | 9.04 | 33.18 | 39.51 | 35.47 | 2.68 |
| | | 30 | 100 | (12) | 0.67 | 8.36 | 35.88 | 33.72 | 21.56 | 4.55 |
| | | 40 | 150 | (12) | 0.89 | 8.92 | 33.63 | 32.70 | 25.34 | 4.17 |
| | | 0 | 50 | (12) | 0.15 | - | | | | 2.48 |

Figure 1. Growth of S. fragilis at different inoculum levels under carbohydrate feed conditions maintaining 5-6% CHO in the broth.

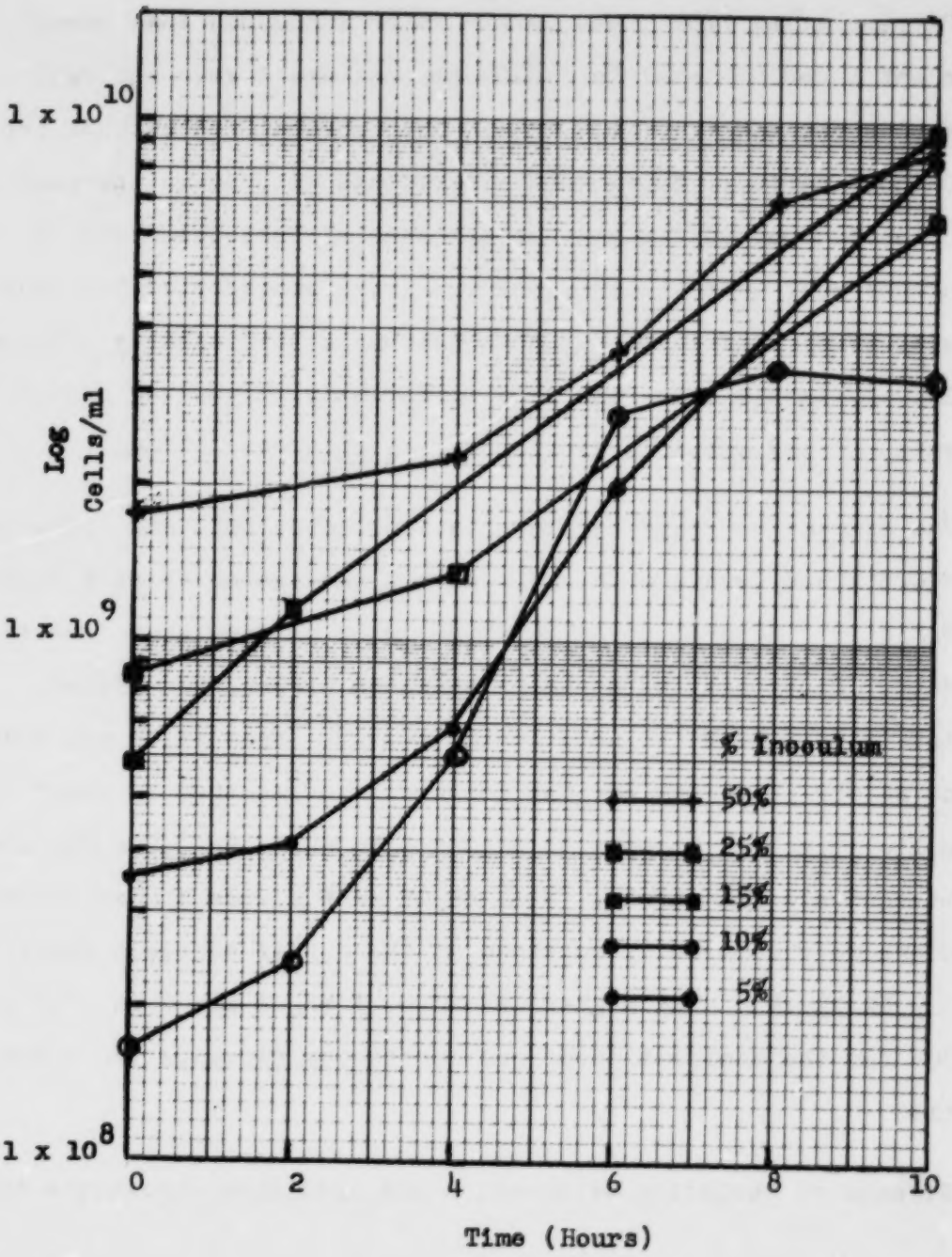


period indicated that doubling time decreased as inoculum size decreased. Thus, inoculum at 44%, 25%, 15%, 10%, 5% had doubling times of 100, 86, 55, 50 and 35 minutes, respectively. Extrapolating from these growth rates indicated that normal plant inoculum levels of 10-20 percent would give the cell yields desired ($>10^9$/ml) in a 8 hour period or 3 batches per 24 hour period. Subsequent experiments without maintenance of 5-6% carbohydrate gave no evidence of inhibition due to excess whey when the 10% inoculum level was adopted for routine use. Whey inhibition was not observed with S. lactis (Figure 2). S. lactis also responded differently in initial growth rate. A lag phase in growth of S. lactis was obvious.

The effects of yeast supplementation (Amber BYF) and different sources and levels of N and P were investigated (Expt's 6-14. Table 1). Several possible trends were extrapolated from the data. First "As Is" proteins appeared higher when higher levels of N were included in the medium. When $NH_4OH$ was used as source of N, the percent conversion of lactose to yeast cellular material appeared higher than when $(NH_4)_2SO_4$ was used at equivalent N levels. With $NH_4OH$ as N source higher conversions were apparently obtained at the higher N-levels (Expt's 8,9,12.13,14). The higher level, 0.9% $NH_4OH$ was then used as SOP for the semicontinuous and continuous fermentation operation.

Effects of variation of aeration and agitation conditions in

Figure 2. Growth of S. lactis from various inoculum levels, grown under conditions of maintenance of 5-6% CHO in the broth.



the fermentor were investigated at standard (6%) and high (11%) carbohydrate levels in the medium with S. fragilis (Expt's 16 and 17, Table 1). These data indicate that the higher aeration conditions 50% Scale Air (≅2.32 V/V/min), 200 rpm to 80% Scale Air, 350 rpm increase conversion efficiency and perhaps % protein in the washed product. They have little beneficial effect on total solids or cell numbers. Conditions below experimental SOP (50% Scale, 250 rpm) result in lower growth and cell yield. The 60% Scale Air, 300 rpm condition would appear optimum and the proper departure point for scaleup to larger equipment.

In order to avoid costly fermentor "downtime" in a short cycle process, the fermentation was tested under semi-continuous (Expt's 11, 15) and continuous (Expt 18) operating conditions. In semi-continuous operation the fermentors were allowed to run until carbohydrate level reached or dropped below 0.5%. At this point 90 percent of the beer was pumped into a holding vessel and an equal amount of fresh medium pumped into the fermentor. In short, 10% of the fermentor beer was retained as inoculum for the next batch. Continuous operation of the fermentation was carried out as described under Materials and Methods. Figure 3 provides data for semi-continuous operation of the process under a high (0.9% $NH_4OH$) and low (0.45% $NH_4OH$) nitrogen condition. Performance for all conditions is for all practical purposes identical and maximum cell growth is obtained in all vessels by 10 hours. In regard to this point, it should

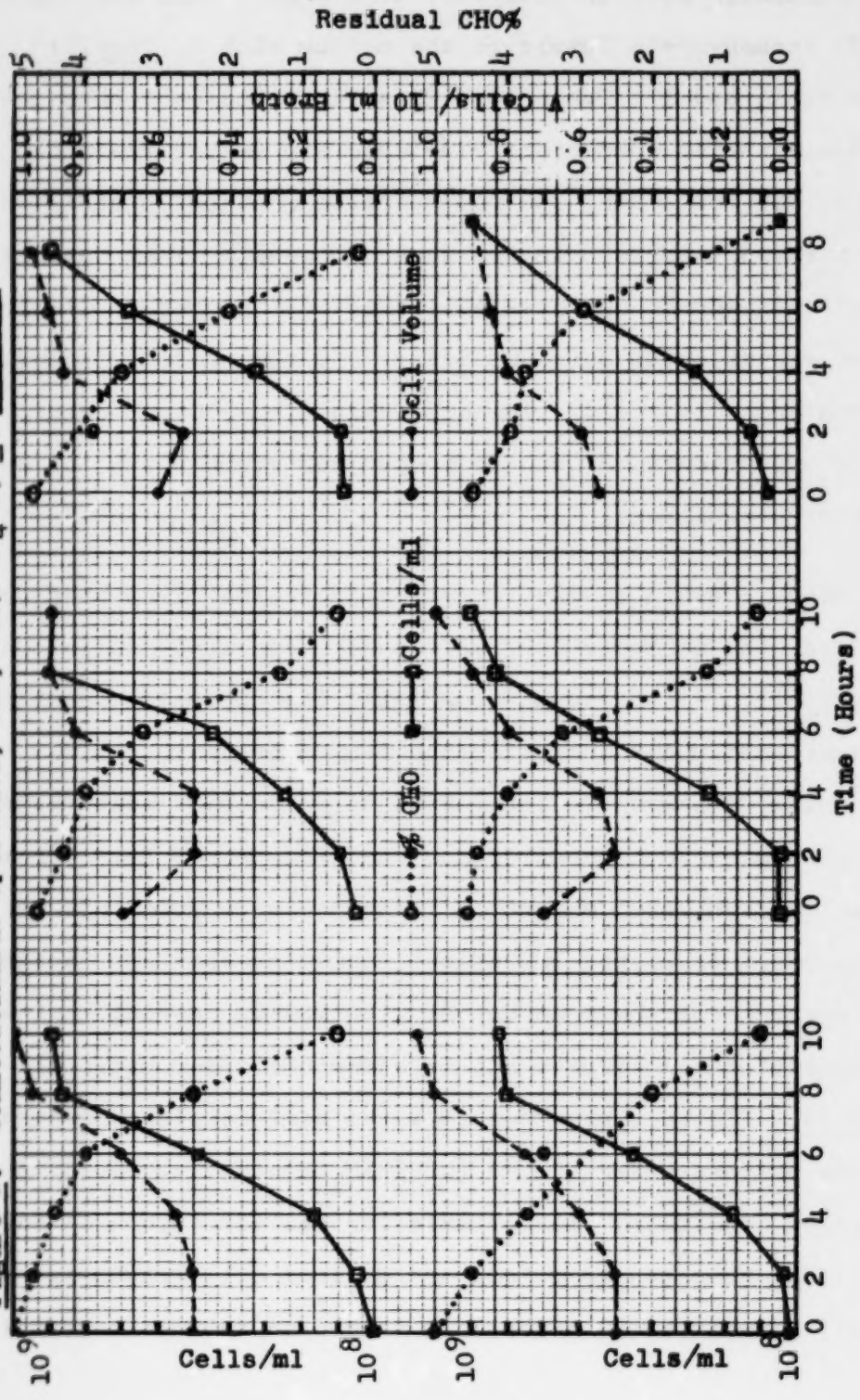



Figure 3. Semicontinuous operation, SOP, 0.9% $NH_4OH$, S. fragilis.

be noted that the trend for the initial vessel set in each series (2,1,4,3) was to reach maximum cell growth at 8 hours while the subcultures were a little slower. This was not due to initial lag. Slightly lower cell levels were reached at 8 hours and the maximum reached between 8 and 10 hours at a lower growth rate. The reason for this is not obvious, but there are sufficient differences in individual subculture growth patterns to prohibit drawing conclusions until the reproducibility of this type of operation is explored further.

The fermentation was observed to operate well in a three-stage continuous system. Data taken on operation of a 60-hour run are given in Table 2 and Figure 4. Medium was fed at a rate of ~3 gal/hr into the first fermentor. This rate is equivalent to 27 gal/9 hr, thus 9 gal/fermentor/9 hr. This approximates batch fermentation time per unit fermentor, but eliminates the fermentor downtime which would have to be taken in batch fermentation operation. The system equilibrated 12 hours after a $1 \times 10^9$ cell population was reached and the initial dilution effect was overcome (22 hours). At equilibrium time the equivalent of 4 batch fermentors had already been harvested. Cell yield out of the third stage fermentor was equivalent to batch level, ca. $1 \times 10^9$ cells/ml, with only minor deviation from this level. About 1-1.9% of ethyl alcohol were accumulated in the third stage of this process. This is lower than the 2-4% levels obtained in batch or semi-continuous operation (Table 1). The effect of process changes on alcohol

TABLE 2. Experiment #18. Continuous operation of a whey fermentation with *S. fragilis* in a 3-stage system

| Vessel | Hours of Operation | Stage #1 Cells/ml x $10^6$ | Stage #1 % Alcohol | Stage #2 Cells/ml x $10^6$ | Stage #2 % Alcohol | Stage #3 Cells/ml x $10^6$ | Stage #3 % Alcohol |
|---|---|---|---|---|---|---|---|
| AI | 0 | 1.26 | | 0.94 | | 0.81 | |
| | 4.5 | 3.6 | | 3.7 | | 3.5 | |
| | 6.0 | 7.0 | | 6.5 | | 5.5 | |
| | 10.0 | 9.7 | | 9.2 | | 10.5 | |
| | 14.0 | 9.1 | | 9.2 | | 10.9 | |
| | 18.0 | 4.7 | | 8.6 | | 12.8 | |
| | 21.0 | 5.0 | | 6.8 | | 12.8 | |
| | 22.0 | 3.5 | | 6.2 | | 11.5 | |
| | 23.0 | 3.9 | | 5.7 | | 13.6 | |
| | 31.0 | 4.1 | 0.33 | 6.8 | 1.0 | 11.4 | 1.27 |
| | 33.0 | 3.8 | 0.43 | 6.2 | 1.09 | 11.5 | 1.45 |
| | 35.0 | 3.8 | 0.54 | 6.3 | 1.18 | 12.5 | 1.44 |
| | 37.0 | 3.4 | 0.34 | 4.7 | .91 | 11.8 | 1.36 |
| | 39.0 | 3.2 | 0.26 | 5.1 | .77 | 12.0 | 1.31 |
| | 41.0 | 3.0 | 0.14 | 4.2 | .30 | 14.0 | 1.30 |
| | 43.0 | 2.6 | 0.11 | 4.2 | .41 | 8.3 | 1.10 |
| | 45.0 | 2.9 | 0.15 | 3.9 | .45 | 9.2 | 1.07 |
| | 47.0 | 2.8 | 0.20 | 4.6 | .45 | 8.6 | 1.05 |
| | 49.0 | 3.1 | 0.73 | 5.7 | 1.05 | 10.6 | 1.90 |
| | 53.0 | 3.5 | - | 5.1 | - | 9.6 | - |
| | 55.0 | 3.4 | 1.09 | 6.5 | 1.48 | 11.2 | 1.79 |
| | 59.0 | 4.2 | 1.23 | 6.4 | 1.83 | 11.5 | 1.97 |
| TERM | 59.7 | 4.8 | 1.55 | 7.3 | 1.84 | 11.3 | 1.97 |

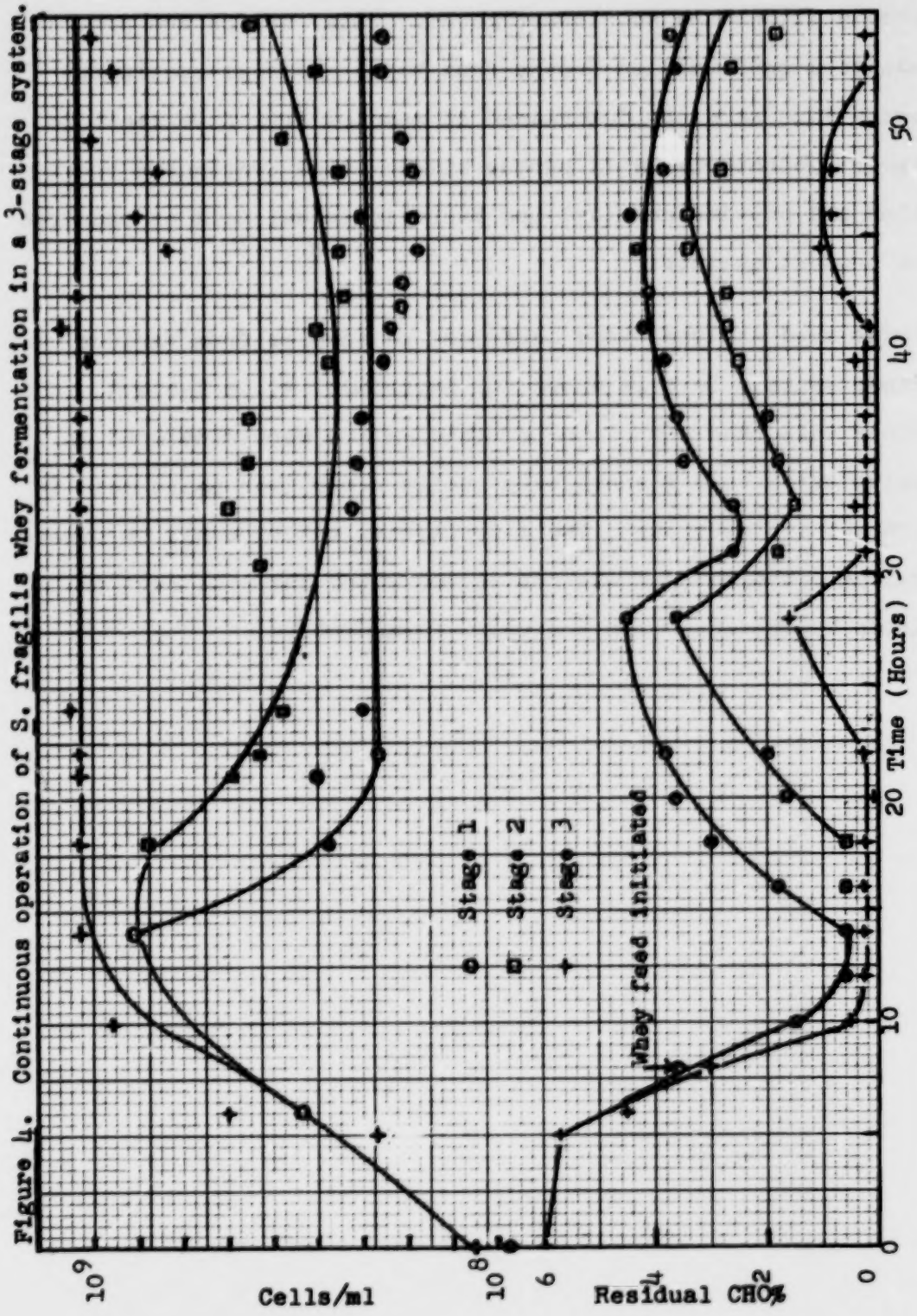



Figure 4. Continuous operation of S. fragilis whey fermentation in a 3-stage system.

accumulation were not investigated. Total solids and protein analyses performed on broths from batch and semi-continuous operations were not determined on samples from Experiment 18. Amber Laboratories will pursue single stage continuous operation and provide chemical and bacteriological data as required on product quality.

Amino acid analyses were performed on samples from various experiments. Data is shown for Experiment 4, in Table 3. Since less than 30% of the protein values were recovered as amino acids some N-containing entity other than amino acid is present in the broth. The N-containing entity could be excess medium nitrogen that was not incorporated into the cell or amide by-products from the yeast fermentation.

TABLE 3. Experiment #4. Amino Acid Analyses, Inoculum Level Effect

| Experimental Conditions | 50% Inoculum | 25% Inoculum | 10% Inoculum | | |
|---|---|---|---|---|---|
| | | | As is | Washed | Enzyme hydrolyzed |
| | | | Amino Acid % | | |
| Lysine | 1.00 | 1.09 | 1.27 | 3.22 | 3.17 |
| Histidine | 0.25 | 0.25 | 0.31 | 0.77 | 0.77 |
| Arginine | 0.36 | 0.42 | 0.51 | 1.48 | 1.76 |
| Aspartic Acid | 1.37 | 1.58 | 1.70 | 0.96 | 3.12 |
| Threonine | 0.77 | 0.87 | 0.96 | 0.48 | 1.54 |
| Serine | 0.79 | 0.85 | 0.93 | 0.51 | 1.61 |
| Glutamic Acid | 3.71 | 2.69 | 3.05 | 1.95 | 4.49 |
| Proline | 1.01 | 1.13 | 1.24 | 2.16 | 1.94 |
| 1/2 Cystine | 0.15 | 0.14 | 0.16 | 0.11 | 0.09 |
| Glycine | 0.49 | 0.44 | 0.50 | 0.55 | 1.25 |
| Alanine | 1.34 | 0.80 | 0.93 | 0.69 | 2.04 |
| Valine | 0.66 | 0.74 | 0.80 | 0.60 | 1.41 |
| Methionine | 0.76 | 0.28 | 0.32 | 0.27 | 0.36 |
| Isoleucine | 0.67 | 0.64 | 0.71 | 0.58 | 1.08 |
| Leucine | 2.22 | 1.27 | 1.43 | 1.70 | 2.31 |
| Tyrosine | 0.37 | 0.41 | 0.48 | 0.74 | 0.92 |
| Phenylalanine | 0.51 | 0.55 | 0.62 | 1.00 | 1.26 |
| | 16.43 | 14.15 | 15.92 | 17.77 | 29.12 |

## SECTION VI

## INTRODUCTION

## AMBER LABORATORIES

The primary goal of the fermentation studies was to develop a method for the production of low cost, single cell protein (SCP). The SCP was produced by Saccharomyces fragilis grown on lactose from whey and to be used for animal feed supplementation. The specific objective of the yeast fermentation studies conducted in the Demonstration Pilot Plant was to scale-up a process Standard-Operating-Procedure (SOP) developed by the subcontractor, International Minerals Corporation (IMC).

The preliminary study conducted by IMC using shake flasks and pilot fermentors produced several conclusions and SOP's for the Amber Laboratories studies. The most encouraging recommendation indicated that the whey fermentation process could be run under non-sterile conditions. In addition preliminary results indicated that operation of the process in a continuous manner might be feasible.

However, the detailed SOP for the continuous process, including product quality, yield and cost estimations required study at Amber Laboratories. The following experiments were conducted to demonstrate a method for simple and economical conversion of lactose from whey into a high quality feed supplement.

## SECTION VII

## MATERIALS AND METHODS

## AMBER LABORATORIES

Saccharomyces fragilis (NURL, Y-1109) was used in the majority of experiments except for two preliminary studies involving Saccharomyces lactis (NURL, Y-1140). Morphologically S. lactis was smaller in size than S. fragilis and it was found that the fermentation broths of S. lactis did not separate efficiently in commercial sized, yeast separators. Therefore, S. lactis was not included in further studies.

In general, concentrated, acid whey was obtained from the manufacture of cream cheese and condensed to 45-50% total solids (T.S.). Acid whey from cream and cottage cheese has pH values in the range of 4.2-4.9 in comparison to sweet whey from Cheddar, Italian, and Swiss cheese with pH values of 5.7-6.2. It was found that the organic nitrogen content of cream cheese whey was lower than other wheys tested. Wasserman (12) found that only 25% of the organic nitrogen could be utilized by S. fragilis, therefore, we assumed that the fermentation characteristics of acid and sweet whey would be similar as long as a slight excess of inorganic nitrogen was added to the media.

The whey was diluted to appropriate levels of lactose with tap water and then used to prepare the various media described below:

Whey agar was prepared for sterile transfers of S. lactis and S. fragilis. Acid whey was diluted to 10% T.S. and the following ingredients added so the media contained (w/v) 0.5% $(NH_4)_2 SO_4$, 0.5% $K_2HPO_4$, and 0.3% Amber BYF 100 (see Glossary). Agar was added to the whey media at a 4% level and the mixture of whey and agar dispensed in suitable aliquots to 20 x 150 mm test tubes. The whey agar tubes were pasteurized at 85-90C for 20 minutes. Saccharomyces lactis and S. fragilis were aseptically transferred on whey agar slants at weekly intervals.

Two basal media were used throughout these studies. SOP Medium-1 was used for inoculum build-up in shake flasks. SOP Medium-2 was used for inoculum build-up in the New Brunswick fermentors and for studies utilizing a 500 gallon, pilot fermentor.

One part whey concentrate was diluted with three parts tap water and the following ingredients added so the medium contained (w/v) 0.5% $(NH_4)_2 SO_4$, 0.5% $K_2HPO_4$ and 0.3% Amber BYF 100. The pH was adjusted to 5.5 for S. lactis and 4.5 for S. fragilis with 85% $H_3PO_4$. The SOP Medium-1 was not sterilized for shake flask use.

Whey concentrate was adjusted to various lactose concentrations by dilution with tap water. The diluted whey was made to contain (w/v) 0.9% $NH_4OH$, 0.3% Amber BYF 100, 0.05% $H_3PO_4$,

and adjusted to pH 5.5 for S. lactis and 4.5 for S. fragilis with 30% HCl. The medium was heated to 80C, held for 45 minutes and cooled. Deviations in the SOP Medium-2 are described in the Experiment Condition for each Experiment Number, (Table 4).

A 500 lb/hour, ultrafiltration (UF) pilot plant manufactured by Abcor. Inc. was utilized to fractionate acid or sweet whey into a protein concentrate and lactose permeate. The lactose permeates were prepared for the fermentation studies by following the SOP Medium-2 format. The permeates were substituted for diluted whey in preparing the media. Analyses of ultrafiltration permeates from both acid and sweet whey were as follows:

| Gross Composition | Ultrafiltration Permeate | |
|---|---|---|
| | Average | Range |
| % Total Solids | 4 | 3-5 |
| % Lactose (dry basis) | 88 | 86-90 |
| % Protein (dry basis) | 4 | 3-5 |
| % Ash (dry basis) | 8 | 7-9 |

The whey permeate was produced from acid or sweet whey using the Abcor ultrafiltration unit (model #UF-80-S) operated under the following conditions:

| Gross Composition of Raw Whey | Acid Whey | Sweet Whey |
|---|---|---|
| pH (as is) | 4.6-4.9 | 5.7-6.0 |
| % Lactic Acid (as is) | 0.50-0.65 | 0.12-0.19 |
| % Total Solids | 6.0-7.0 | 5.5-6.5 |
| % Protein (dry basis) | 5.0-9.0 | 12.5-14.0 |
| % Ash (dry basis) | 8.0-12.5 | 6.0-8.0 |

Operating Conditions

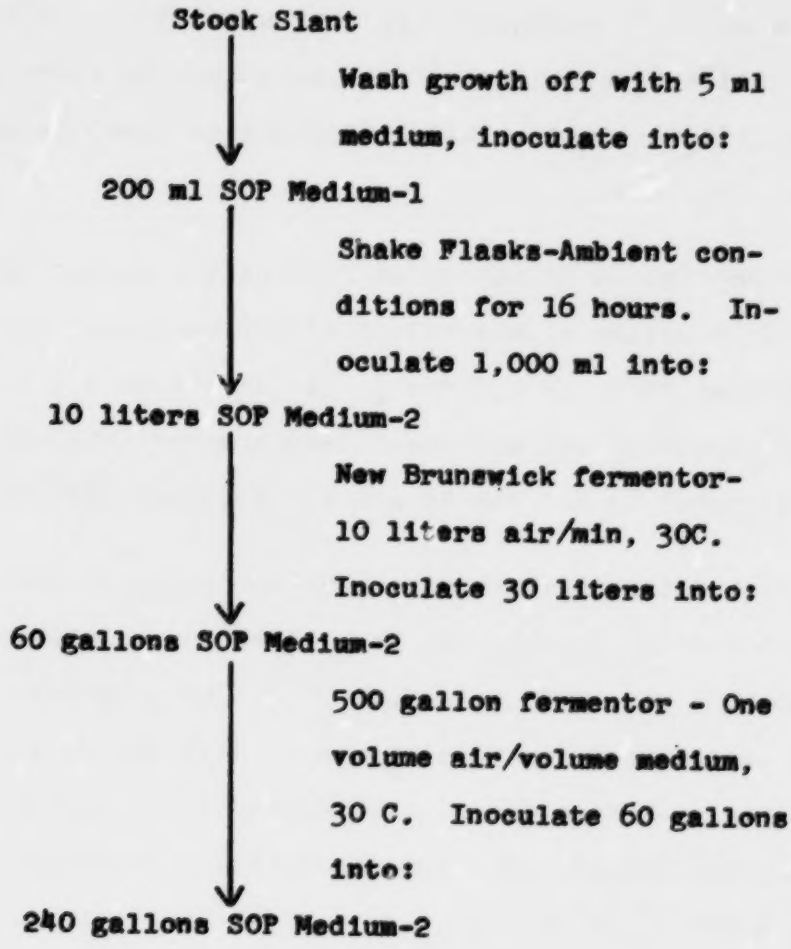
| | |
|---|---|
| Inlet Temp. of Whey | 120-124°F |
| Inlet Pressure | 55 PSIG |
| Outlet Pressure | 25 PSIG |
| Flux Rate | 2.0-3.0 gal/hr |
| Membrane Surface Area | 70 $ft^2$ |

The closed-loop fermentation studies were made utilizing media prepared according to the SOP Medium-2 format. However condensate water was substituted for tap water to dilute the whey concentrate. The condensate water, derived from the evaporation process of fermentation broths, contained up to 4% alcohol (w/v) and less than one part per million nitrogen.

A 500 gallon, fully baffled, deep tank fermentor was used for the semi-continuous and continuous studies. The media were aerated by compressed air entering through a bottom sparger and agitated by a standard shaft and impeller system. (See Appendices for photographs of equipment). Constant temperature control of the media was obtained by circulation of water and steam through the jacket of the fermentor.

Three 15-liter New Brunswick fermentors served as the seed vessels for the 500 gallon fermentor. The New Brunswick fermentors were manufactured to standard design and contained air agitation and temperature controls.

Inoculum for the 500 gallon fermentor was prepared according to the following procedure:


Stock Slant
Wash growth off with 5 ml medium, inoculate into:
200 ml SOP Medium-1
Shake Flasks-Ambient conditions for 16 hours. Inoculate 1,000 ml into:
10 liters SOP Medium-2
New Brunswick fermentor-10 liters air/min, 30C. Inoculate 30 liters into:
60 gallons SOP Medium-2
500 gallon fermentor - One volume air/volume medium, 30 C. Inoculate 60 gallons into:
240 gallons SOP Medium-2

The semi-continuous fermentations were made by drawing off 90% of the fermentation broth and using the remaining 10% to seed the next fermentation batch. The number of consecutive draw-down batches are indicated in each experiment (Table 4). The continuous fermentations were begun when the cell count of the fermentation broth reached 1 x $10^9$/ml and the lactose concentration, 0.50-0.75% (w/v). The rate of continuous feed and removal was maintained at 37 gallons/hour which corresponded

to a batch fermentation cycle of about 300 gallons/8 hours (dilution rate 0.125 $hr.^{-1}$). The number of hours the fermentation was maintained continuous is shown in each experiment (Table 4).

The conditions of operation for the 500 gallon fermentor were 0.5-1.0 volume of air/volume of SOP Medium-2; agitator speed 200 rpm; 30°C; pH 5.5 for S. lactis and pH 4.5 for S. fragilis. The fermentor was not sterilized however, the SOP Medium-2 was heated to 80C for 45 min as described earlier.

Solids determinations were made according to the Mojonnier Method (7). Protein was determined in accordance with the A.O.A.C. Method (1). A factor of 6.25 x percent - N was used as recommended for yeast protein. Ash was determined in accordance with the A.O.A.C. Method (1). Lactose concentration was determined using the Anthrone procedure described by Umbreit, et al. (9). Amino Acid analyses performed by Wisconsin Alumni Research Foundation using the method described by Moore, et al. (8). Official rat Protein Efficiency Ration assay performed by Wisconsin Alumni Research Foundation using the A.O.A.C. procedure (1). Cell count analyses made according to International Mineral Corporation Method (3). Results reported as yeast cells/ml of fermentation broth.

## SECTION VIII

## RESULTS AND DISCUSSION

## AMBER LABORATORIES

The effects of variation in the type of acid (phosphoric or hydrochloric) used to adjust the SOP Medium-2 to pH 4.5 on yeast cell population, gross composition of yeast and conversion of lactose to yeast material are shown in Experiment Numbers 1-7, (Table 4). The use of phosphoric acid to adjust the pH of the medium prior to and during fermentation produced yeasts with ash levels of 35-43%. The inclusion of hydrochloric acid (HCl) in the medium resulted in a lower ash content that approximated the 9-13% ash level typical of commercial yeasts. The use of low cost HCl did not adversely affect the fermentation and produced a more economical product than that obtained with phosphoric acid.

The 0.9% (w/v) ammonium hydroxide level recommended by IMC investigators and used in Experiments 1-7, 15 and 18, (Table 4) as a supplementary nitrogen source, resulted in S. fragilis yeasts with crude protein levels that were abnormally high. The effects of 0.3-0.7% (w/v) ammonium hydroxide concentrations on growth of S. fragilis are shown in Experiments 8-13, (Table 4). It was found that the crude protein levels approximated commercial yeasts with ammonium hydroxide levels of 0.3-0.7% and the conversion of lactose to yeast material remained constant with varied ammonium hydroxide levels. A comparison of net protein concentrations ((% crude nitrogen-

Table 4. Summary of Fermentation Experiments
EPA Project S-800747

| Exp. Number | Experiment Condition | Lactose Conc. | Max. Pop. Cells/ml X $10^9$ | T.S. FWM | % Dry Basis | | % Conversion gm T.S./ gm lactose |
|---|---|---|---|---|---|---|---|
| | | | | | Crude Protein | Ash | |
| 1 (4)[a] | S. fragilis Semi-continuous SOP Medium-2 Phosphoric Acid Acid Whey 6-300 gal. Tank Ferm. | 7-8% | 0.55 | 7.1 | 43.4 | 35.0 | 0.96 |
| 2 (5) | S. fragilis Semi-continuous SOP Medium-2 Phosphoric Acid Acid Whey 8-300 gal. Tank Ferm. | 7-8% | 0.75 | 7.9 | 48.6 | 42.6 | 1.01 |
| 3 (2) | S. fragilis Batch SOP Medium-2 Acid Whey 1-300 gal. Tank Ferm. | 6-7% | 0.30 | 5.5 | 56.8 | 20.9 | 0.85 |

[a] Numbers in parenthesis refer to raw data experiments

Table 4 (cont.)

| Exp. Number | Experiment Condition | Lactose Conc. | Max. Pop. Cells/ml X $10^9$ | T.S. FWM | % Dry Basis Crude Protein | % Dry Basis Ash | % Conversion gm T.S./ gm lactose |
|---|---|---|---|---|---|---|---|
| 4 (6) | S. fragilis Semi-continuous SOP Medium-2 Acid Whey 7-300 gal. Tank Ferm. | 8-9% | 1.52 | 8.2 | 73.5 | 15.6 | 0.96 |
| 5 (13) | S. fragilis Continuous SOP Medium-2 Acid Whey Ferm. Operated 59 hr. cont. | 5-6% | 2.50 | 5.1 | 76.9 | 17.2 | 0.92 |
| 6 (15) | S. fragilis Continuous SOP Medium-2 Acid Whey Ferm. Operated 90 hr. cont. | 5-6% | 2.50 | 5.19 | 69.7 | 15.2 | 0.99 |
| 7 (16) | S. fragilis Continuous SOP Medium-2 Acid Whey Ferm. Operated 220 hr. cont. | 5-6% | 2.24 | 5.89 | 68.5 | 17.6 | 0.93 |

Table 4 (cont.)

| Exp. Number | Experiment Condition | Lactose Conc. | Max. Pop. Cells/ml X $10^6$ | T.S. FWM | % Dry Basis Crude Protein | % Dry Basis Ash | % Conversion gm T.S./ gm Lactose |
|---|---|---|---|---|---|---|---|
| 8 (9) | S. fragilis Semi-continuous SOP Medium-2 0.7% $NH_4OH$ Acid Whey 8-300 gal. Tank Ferm. | 5-6% | 1.22 | 5.38 | 66.1 | 13.2 | 1.08 |
| 9 (10) | S. fragilis Semi-continuous SOP Medium-2 0.7% $NH_4OH$ Acid Whey 6-300 gal. Tank Ferm. | 8-9% | 1.57 | 8.31 | 51.7 | 13.9 | 0.96 |
| 10 (7) | S. fragilis Semi-continuous SOP Medium-2 0.5% $NH_4OH$ Acid Whey 8-300 gal. Tank Ferm. | 4-5% | 0.88 | 4.98 | 47.9 | 22.8 | 1.15 |

Table 4 (cont.)

| Exp. Number | Experiment Condition | Lactose Conc. | Max. Pop. Cells/ml X $10^9$ | T.S. FWM | % Dry Basis Crude Protein | % Dry Basis Ash | % Conversion gm T.S./ gm lactose |
|---|---|---|---|---|---|---|---|
| 11 (8) | S. fragilis Semi-continuous SOP Medium-2 0.5% $NH_4OH$ Acid Whey 10-300 gal. Tank Ferm. | 4-5% | 1.21 | 4.52 | 54.2 | 14.0 | 0.91 |
| 12 (21) | S. fragilis Continuous SOP Medium-2 0.3% $NH_4OH$ Acid Whey Ferm. Operated 64½ hr. cont. | 4-5% | 2.17 | 5.13 | 44.6 | 11.2 | 1.11 |
| 13 (26) | S. fragilis Continuous SOP Medium-2 0.3% $NH_4OH$ Acid Whey Ferm. Operated 56 hr. cont. | 7-8% | 2.16 | 4.94 | 32.8 | 25.9 | 0.68 |

Table 4 (cont.)

| Exp. Number | Experiment Condition | Lactose Conc. | Max. Pop. Cells/ml X $10^9$ | T.S. FWM | % Dry Basis Crude Protein | % Dry Basis Ash | % Conversion gm T.S./ gm lactose |
|---|---|---|---|---|---|---|---|
| 14 (25) | S. fragilis Continuous SOP Medium-2 Acid Whey - Permeate 0.3% $NH_4OH$ Ferm. Operated 50 hr. cont. | 3-4% | 2.20 | 3.04 | 51.6 | 16.6 | 0.83 |
| 15 (14) | S. fragilis Semi-continuous SOP Medium-2 Sweet Whey 7-300 gal. Tank Ferm. | 4-5% | 1.54 | 5.38 | 81.0 | 11.8 | 1.26 |
| 16 (22) | S. fragilis Continuous SOP Medium-2 Sweet Whey 0.3% $NH_4OH$ Ferm. Operated 76 hr. cont. | 6-7% | 2.43 | 5.38 | 52.2 | 14.5 | 0.82 |

Table 4 (cont.)

| Exp. Number | Experiment Condition | Lactose Conc. | Max. Pop. Cells/ml X $10^9$ | T.S. FWM | % Dry Basis Crude Protein | % Dry Basis Ash | % Conversion gm T.S./ gm lactose |
|---|---|---|---|---|---|---|---|
| 17 (24) | S. fragilis Continuous SOP Medium-2 Sweet Whey-Permeate 0.3% $NH_4OH$ Ferm. Operated 32 hr. cont. | 3-4% | 1.91 | 3.31 | 63.9 | 11.7 | 0.88 |
| 18 (12) | S. fragilis Semi-continuous SOP Medium-2 Acid Whey Closed Loop Condensate - 4.1% w/v Ethyl Alcohol 5-300 gal. Tank Ferm. | 4-5% | 1.01 | 5.43 | 65.1 | 9.6 | 1.21 |
| 19 (27) | S. fragilis Continuous SOP Medium-2 Acid Whey 0.3% $NH_4OH$ Closed Loop Condensate-0.8% w/v Ethyl Alcohol Ferm. Operated 38 hr. cont. | 6-7% | 2.11 | 4.83 | 40.3 | 18.4 | 0.70 |

% ammoniacal nitrogen) x 6.25)) was made between the whole fermented whey mass from a media with 0.3-0.7% ammonium hydroxide and a media with 0.9% ammonium hydroxide. The net protein values were 30-35% for both levels of supplemented nitrogen. It was apparent that the 0.9% ammonium hydroxide level did not increase the nitrogen up-take into cellular protein. Therefore, ammonium hydroxide levels of 0.3-0.7% (w/v) were used for production of S. fragilis yeast on whey.

Although lactose derived from acid whey was used throughout the studies as the main carbon source for yeast fermentation experiments were performed using lactose from sweet whey (Experiments 15-16, & 17, Table 4). No significant differences in fermentation characteristics were noted between acid and sweet whey (compare Experiment 4 (Acid Whey) with Experiment 15 (Sweet Whey) and Experiment 12 (Acid Whey) with Experiment 16 (Sweet Whey)).

A corollary to the fermentation study of acid and sweet whey, was to study the fermentation characteristics of deproteinated acid or sweet whey from an UF unit. The results shown in Experiments 14 and 17, Table 4, indicated that yeast would ferment lactose from an ultrafiltration permeate and produce similar cell counts and crude protein concentrations as yeast propagated on whole acid or sweet whey. Under the conditions of these experiments a number of difficulties were experienced in using ultrafiltration as a pre-treatment for acid or sweet

whey to be used for fermentation. The raw material used for the UF unit was whey (acid or sweet) having a solids content of 5.5-7.0%. The resultant permeate was very low in solids (total solids 3-5%) which would be uneconomical to ferment. If the solids of the raw whey were raised by evaporation or partial dilution of condensed whey, considerable membrane fouling and clean-up problems were experienced. Operation of the UF unit under these conditions reduced flux rates by 50-75%. In such a situation, mechanical cleaning (with a sponge ball) of the membranes was necessary which probably decreased membrane life. Moreover, the inability to adequately sanitize the UF unit resulted in extremely high plate counts after completion of each experiment. Therefore, it was concluded that the use of UF pre-treatment of whey prior to fermentation would be unattractive.

A study of the feasibility of using a method for minimizing waste streams from the fermentation operation was conducted in Experiments 18 and 19, (Table 4). The block diagram describing the closed-loop system is shown in Figure 5. A closed loop design was used which consisted of dilution of acid whey concentrate with condensate water derived from the evaporation process of fermentation broths followed by fermentation of the whey. Theoretically, the closed-loop could be repeated as often as new whey concentrate was added to the cycle. Since the entire condensed fermentation broths were spray dried, no waste streams would be obtained from the process.

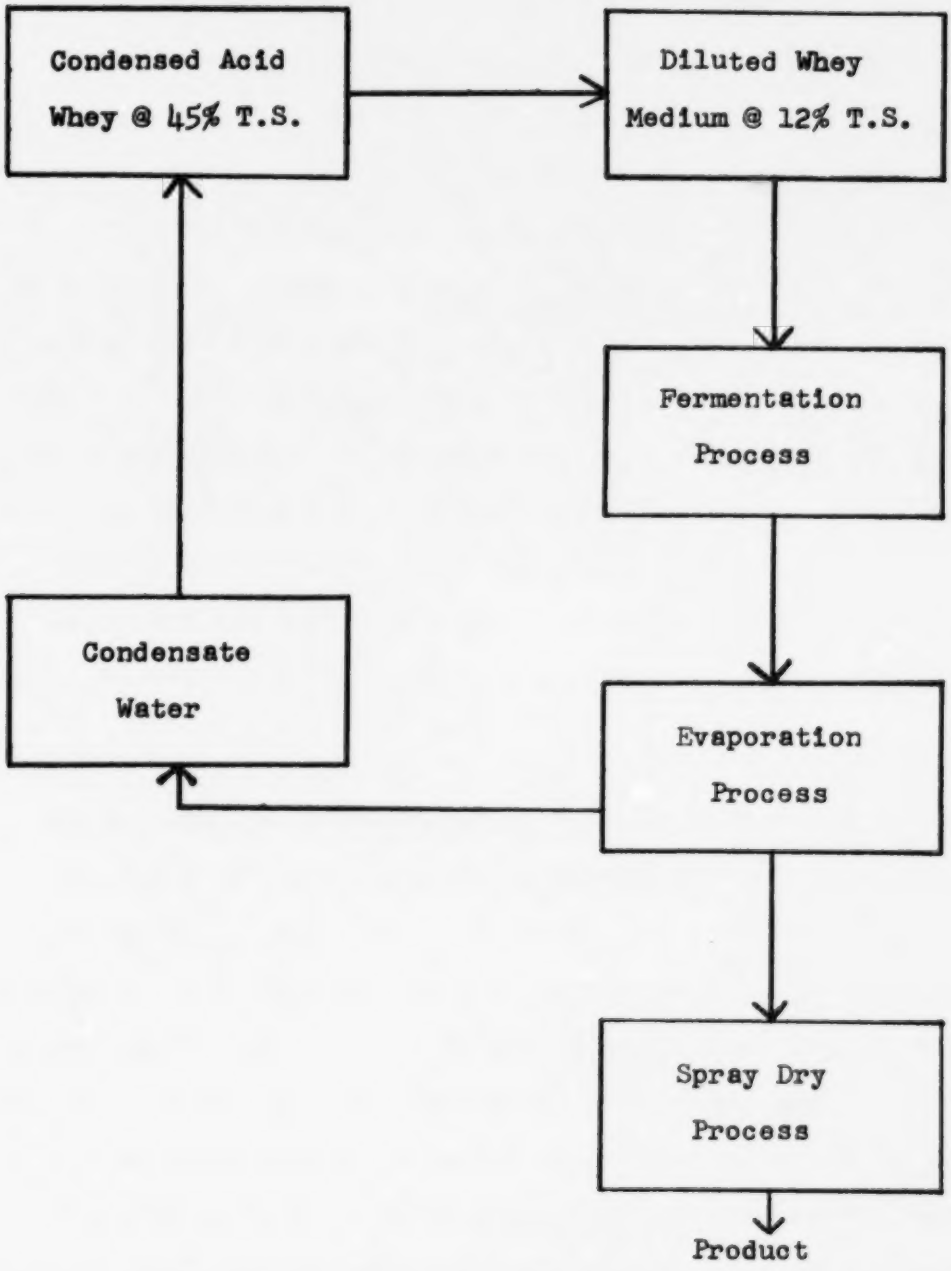



FIGURE 5. BLOCK DIAGRAM OF CLOSED-LOOP SYSTEM FOR MINIMIZING FERMENTATION EFFLUENTS

The fermentation results were not significantly different from acid whey fermentations using tap water for dilution although ethyl alcohol concentrations as high as 4% (w/v) were present in the condensate water.

Although the emphasis of the studies was to develop a fermentation method for production of high quality feed supplements, several yeasts were washed to produce food yeasts. A bland food yeast was obtained with crude protein concentrations of 52-55% and ash concentrations of 6-10%. If the higher grades of yeast products are produced, the additional processing through centrifugation produced a supernatant effluent that would have to be processed further by some form of waste treatment. These supernatant streams from the centrifuges contained 5 day BOD values that averaged 10,100 mg/l (average for 16 experiments, range 5100-12,700 mg/l). The principal organic materials in these streams were ethyl alcohol (average less than 2% w/v) and lactose (average 0.5%). These supernates could not be used to dilute incoming condensed whey (as in the closed-loop process) because high concentrations of inorganic salts were present in the supernatant which interfered with the fermentation. If a centrifuged product was desired, it was found that yeast slurries could be centrifuged successfully after partial evaporation (to 15-17% T.S.) which reduced supernate volume substantially without raising the BOD value (still 10,000 mg/l). The BOD value did not increase because some of the ethanol was removed

during partial evaporation.

Throughout the entire study, non-sterile, semi-continuous, and continuous operation of the fermentation process was demonstrated. The results shown in Figures 6, 7, and 8 are typical of the high cell populations ($1 \times 10^9$ cells/ml) obtained with S. fragilis using the SOP Medium-2.

A comparison of the gross composition and amino acid content of several single-cell proteins is presented in Table 5. Another comparison of the amino acid composition of several yeasts, whole wheat, and the FAO (FAO Committee, World Health Organization, United Nations) profile is shown in Table 6. The high lysine and threonine contents of S. fragilis and commercial Brewers' yeast and low values for wheat make the yeasts valuable supplements for cereal diets. The low content of sulfur amino acids, especially methionine, in S. fragilis and commercial Brewers' yeast is an obvious deficit for a feed supplement. It has been reported by Kihlberg (4) that yeast with added methionine produced a high quality feed supplement.

Feeding studies with growing rats were conducted on centrifuged (commercial, nozzle type, yeast centrifuges) fermented whey mass (FWM) and whole FWM which was fed as the only nitrogen source in an otherwise adequate diet. The results are reported in Table 7 and plotted in Figure 9. The Protein Efficiency Ratio (PER) of centrifuged, FWM was greater than

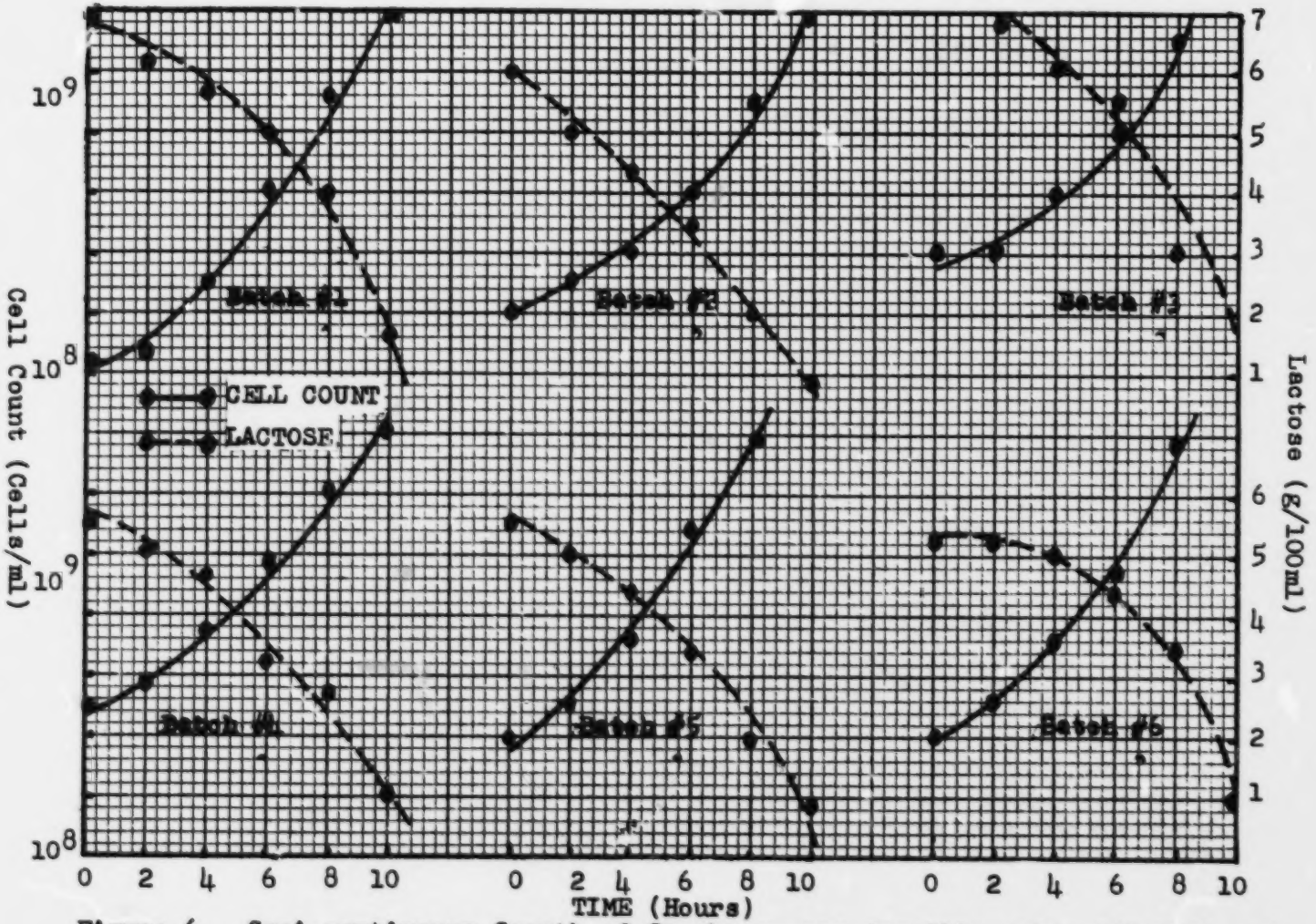



Figure 6. Semi-continuous Growth of Saccharomyces fragilis using SOP Medium-2.

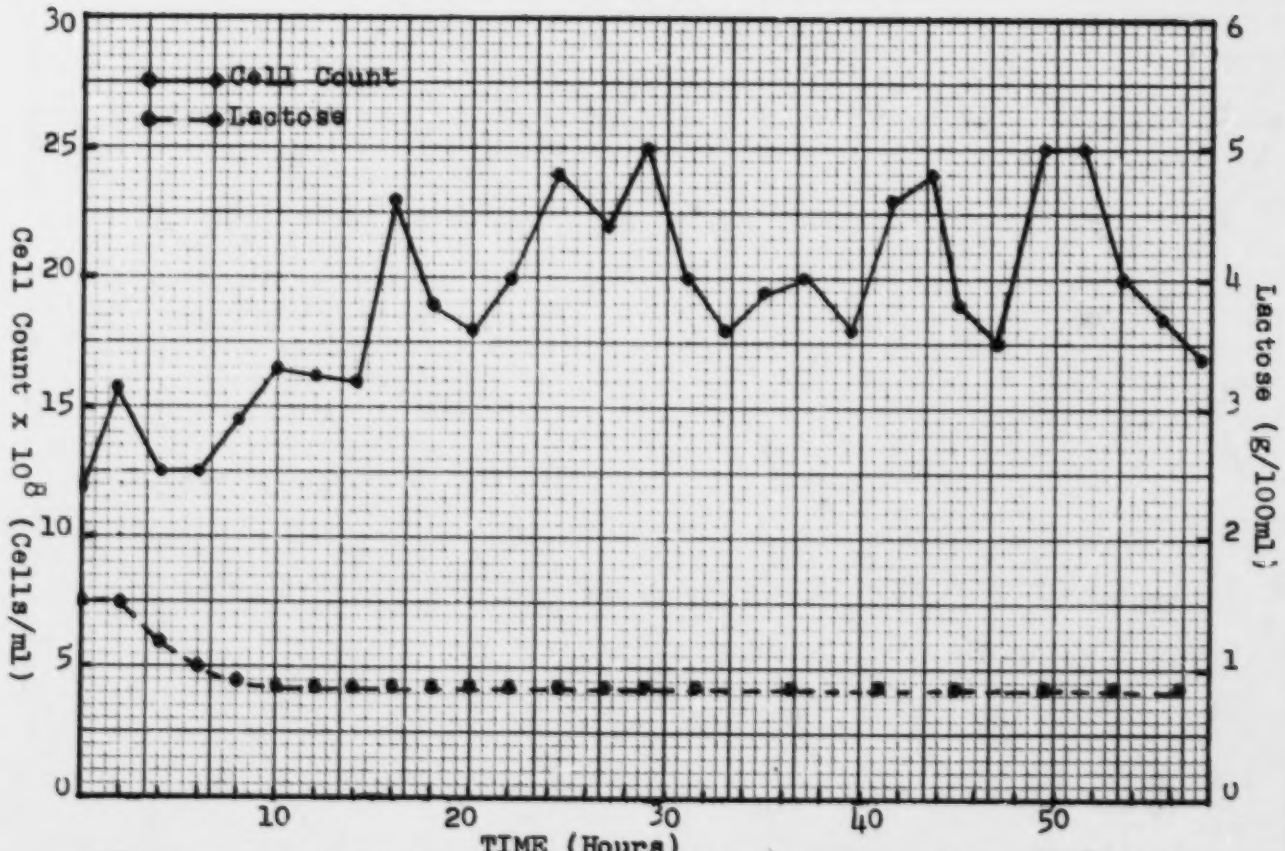



Figure 7. Continuous Growth of Saccharomyces fragilis Using SOP Medium-2 (59 Hr.).

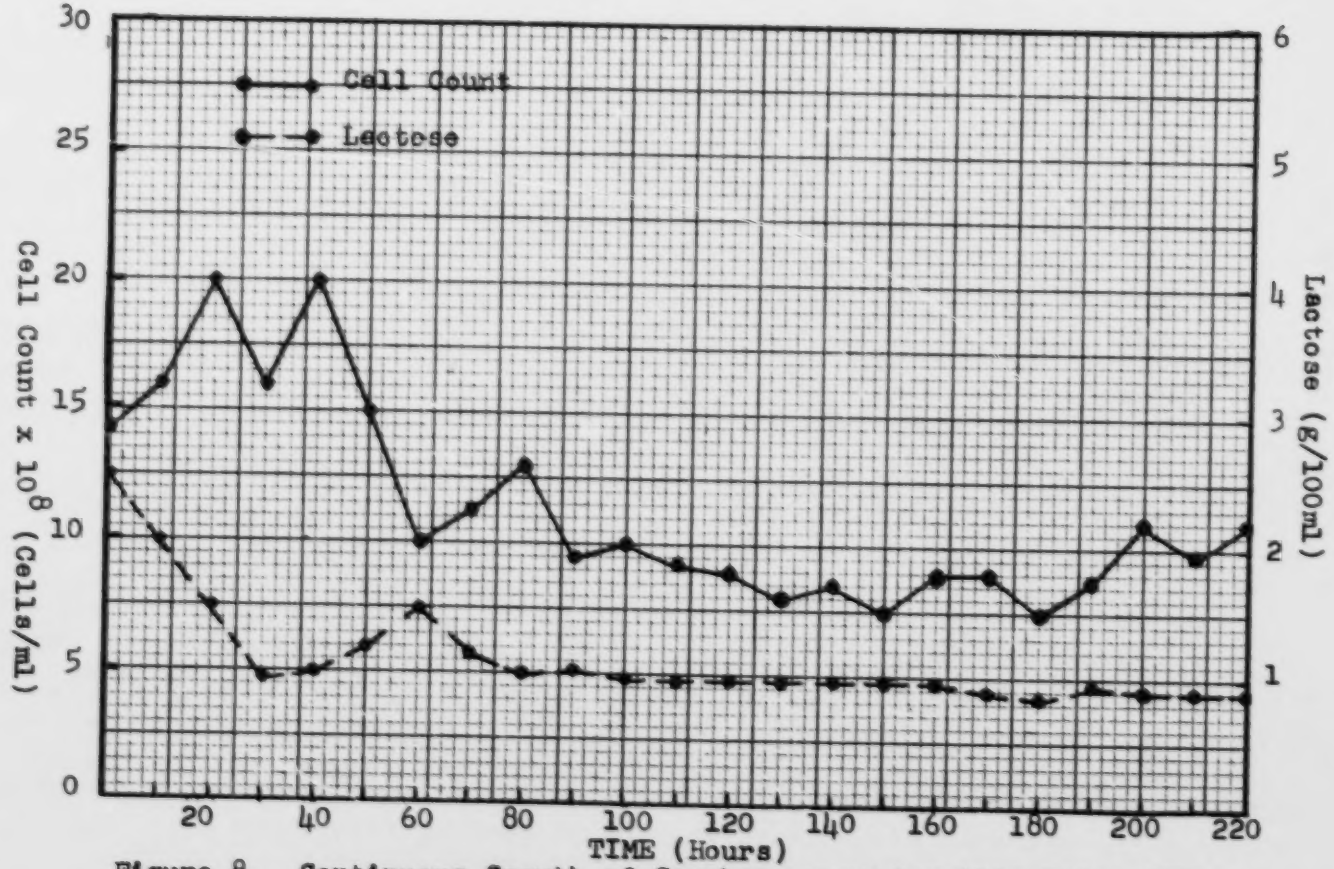



Figure 8. Continuous Growth of Saccharomyces fragilis Using SOP Medium-2 (220 Hr.).

Table 5. Comparison of Gross Composition and Amino Acid Content of Various Single-Cell Proteins

| | Esso Yeast (10) | Wheast (5) | Brewers Yeast | Torula Yeast | S. Fragilis FWM | |
|---|---|---|---|---|---|---|
| Typical Analysis | | | % (As Is) | | | |
| Protein | 54.0 | 57.2 | 44.9 | 47.0 | 44.6 | |
| Fat | 10.0 | 1.1 | 0.7 | 1.2 | 1.1 | |
| Ash | 10.0 | 8.5 | 6.9 | 6.9 | 11.2 | |
| Moisture | [a] | 5.0 | 3.0 | 5.0 | 3.5 | |
| Amino Acid | | | % of Total Protein | | | |
| Lysine | 7.0 | 7.4 | 6.8 | 8.5 | 6.9 | [b] (8.8) |
| Threonine | 3.9 | 5.2 | 5.9 | 5.1 | 5.8 | (5.5) |
| Methionine | 1.2 | 1.5 | 1.5 | 1.5 | 1.9 | (1.5) |
| Valine | 4.0 | 6.3 | 4.7 | 5.6 | 5.4 | (6.6) |
| Leucine | 5.9 | 7.6 | 5.8 | 8.0 | 6.1 | (9.9) |
| Isoleucine | 3.6 | 5.2 | 3.6 | 6.4 | 4.0 | (5.5) |
| Tyrosine | [a] | 3.1 | 2.7 | 4.3 | 2.4 | [a] |
| Phenylalanine | 3.7 | 3.7 | 3.4 | 5.1 | 2.8 | (3.9) |
| Tryptophan | 0.5 | 1.4 | 1.1 | 1.1 | 1.0 | (1.5) |

[a] Not Reported

[b] Dried Fragilis Yeast, Standard Brands Inc., 1967 (6)

Table 6. Amino Acid Content of Whole Wheat, Commercial Brewers Yeast and *Saccharomyces fragilis* Yeast compared to FAO Profile

| Amino Acid (7) | FAO Profile | Whole Wheat (4) | Brewers Yeast | *S. fragilis* Expt. #5 |
|---|---|---|---|---|
| | % of Total Protein | | | |
| Lysine | 4.2 | 2.8 | 6.8 | 6.9 |
| Threonine | 2.8 | 2.9 | 5.9 | 5.7 |
| Methionine | 2.2 | 1.5 | 1.5 | 1.9 |
| Valine | 4.2 | 4.4 | 4.7 | 5.4 |
| Leucine | 4.8 | 6.7 | 5.8 | 6.1 |
| Isoleucine | 4.2 | 3.3 | 3.6 | 4.0 |
| Tyrosine | 2.8 | - | 2.7 | 2.4 |
| Phenylalanine | 2.8 | 4.5 | 3.4 | 2.8 |
| Tryptophan | 1.4 | 1.1 | 1.1 | 1.0 |
| Histidine | - | - | 2.1 | 2.1 |
| Arginine | - | - | 7.4 | 3.7 |
| Aspartic Acid | - | - | 8.3 | 8.6 |
| Serine | - | - | 4.3 | 4.1 |
| Glutamic Acid | - | - | 12.1 | 13.4 |
| Proline | - | - | 4.2 | 4.1 |
| Glycine | - | - | 3.9 | 3.6 |
| Alanine | - | - | 5.7 | 5.7 |
| Cysteine | 2.0 | 2.5 | [a] | [a] |

[a] Not Reported

Table 7. Protein Efficiency Ratio (PER) Assays of Several Typical *Saccharomyces fragilis* Yeasts.

| Sample | Average Weight Gain - gm | | | | |
|---|---|---|---|---|---|
| | 1 week | 2 week | 3 week | 4 week | Total |
| Fermented Whey Mass Experiment #16 Centrifuged, | 3 | 15 | 18 | 17 | 53 |
| Fermented Whey Mass Experiment #12 | 18 | 25 | 22 | 27 | 92 |
| ANRC Casein | 20 | 24 | 25 | 31 | 100 |

| Sample | Average Protein Consumed - gm | | | | |
|---|---|---|---|---|---|
| | 1 week | 2 week | 3 week | 4 week | Total |
| Fermented Whey Mass Experiment #16 Centrifuged, | 3.6 | 7.1 | 8.1 | 8 1 | 26.9 |
| Fermented Whey Mass Experiment #12 | 5.2 | 7.7 | 10.5 | 12 1 | 35 5 |
| ANRC Casein | 5.4 | 7.7 | 9.7 | 12.2 | 35.0 |

| Sample | Average PER Value - 4 week | |
|---|---|---|
| | As is | Corrected[1] |
| Fermented Whey Mass Experiment #16 Centrifuged, | 1.97 | 1.72 |
| Fermented Whey Mass Experiment #12 | 2.59 | 2.26 |
| ANRC Casein | 2.86 | 2.50 |

[1] Corrected to 2.50 as PER value of Casein
(as is value) x (2.50/2.86) = Corrected

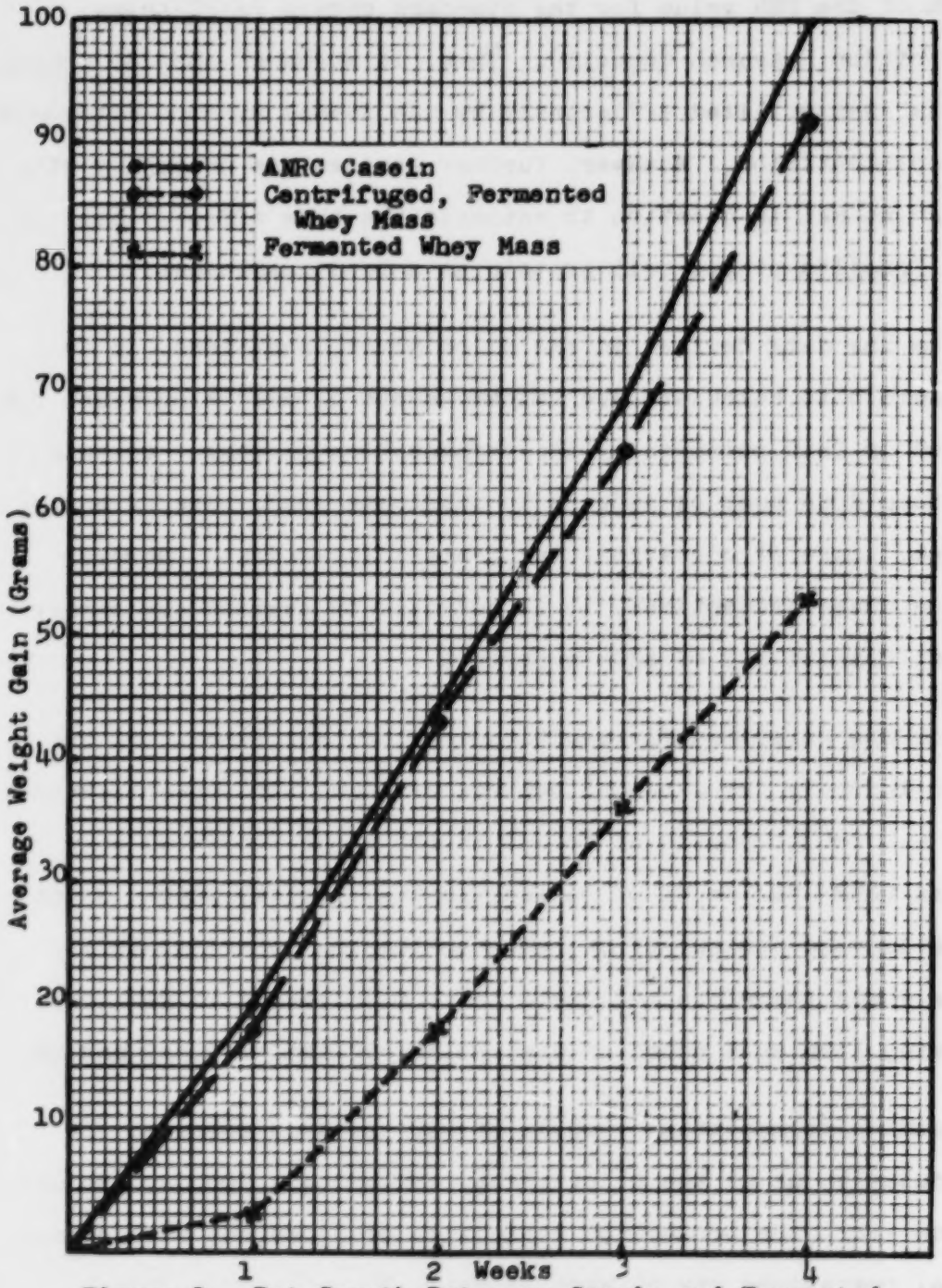



Figure 9. Rat Growth Rates on Casein and Fermented Whey Products.

90% of the PER value for the standard casein (ANRC-Animal Nutrition Research Council). Thus, it appeared that S. fragilis yeast was utilized efficiently by the animal without methionine supplementation. However, further studies are necessary with each animal in question to estimate the true effectiveness of S. fragilis single cell protein as a feed supplement.

From the data derived in the pilot plant operations, it is possible to calculate preliminary costs of producing yeast for food or feed purposes by the fermentation of whey. Certain assumptions must be made as to substrate cost, size of equipment, hours of operation, and capital investment. However, from these assumptions, it is possible to examine the commercial feasibility of such a process.

From the experimental results in the 500 gal fermentor, run continuously for extended periods of time, the conversion rate is 0.9 to 1.0 pounds of solids in the whole fermented whey mass (FWM) per pound of lactose in the original whey. Since the lactose content of the whey solids is approximately 75% one would therefore realize 0.68 to 0.75 pounds of FWM (dry basis) from each pound of whey solids in the original medium.

Assuming fermentation vessels of various size operated continuously, as in the pilot plant, and using a 300 day production year, the production capacities of different size commercial operations may be seen in Table 8.

Table 8. Annual Production of Fermented Whey Mass Solids vs Fermentor Size

| Size of Fermentor (gallons) | Fermented Whey Mass Solids (Tons/yr) |
|---|---|
| 5,000 | 900 |
| 10,000 | 1,800 |
| 20,000 | 3,600 |
| 30,000 | 5,400 |
| 40,000 | 7,200 |

The cost of the medium for the fermentation is based on a 10-12% total solids level and will be primarily determined by the price of whey. While it is not difficult to foresee a time when this material will have a negative value due to the necessary cost of waste treatment and pollution abatement, at the present time it may be possible to obtain the needed whey for no cost or for the cost of transportation only. Using a cost of whey of zero as a low value and 2¢/pound solids as a high value, medium costs will be in the range of 2.5-5.7¢/ pound of product.

The cost of production of FWM material is directly affected by the size of the operation. The cost of labor and operation decreases on a per pound, finished product basis as the

size of the equipment and its degree of sophistication increases. However, as the capacity increases so does the capital investment with its connected charges for depreciation, taxes, insurance and physical facilities.

A plant capable of an annual production of 4,000 to 10,000 tons per year would cost 5 to 15 million dollars depending on the design. A plant this size is considered a small fermentation plant, in light of those being designed for the production of single cell protein from hydrocarbons (100,000 ton, annual capacity).

The operating costs of a yeast-whey fermentation, including those for utilities, power, aeration, recovery and processing are lower than other processes for producing similar materials from other substrates. The aeration requirements for the fermentation of hydrocarbons is 2.5 to 5.0 times that of the fermentation of a carbohydrate such as lactose (10). Because of the solubility of the whey substrate, agitation and power needs are lower, as is the amount of cooling. A cost comparison between acid whey, hydrocarbon, and cellulose fermentations is shown in Table 9. The basis of the production capacities are 100,000 tons per year for the products from hydrocarbons or cellulose raw material and 4,000-8,000 tons per year for FWM from whey.

Table 9. Production Cost Estimates of Various Single-Cell Proteins

| Type of Cost | Acid Whey | Hydrocarbon (10) | Cellulose (2) |
|---|---|---|---|
| | | Annual Capacity (Tons) | |
| | 4,000-8,000 | 100,000 | 100,000 |
| | | Cents per pound | |
| Medium | 2.5-5.7 | 2.0-4.0 | 3.0 |
| Operating & Utilities | | | |
| Aeration & Agitation | 0.50 | 1.25-2.00 | 0.50 |
| Cooling | 0.50 | 1.00 | 0.10 |
| Recovery | 0.30 | 0.25-0.50 | 0.20 |
| Drying | 0.90 | 0.40-0.60 | 0.50 |
| Other | 0.60 | 0.20-0.40 | 0.30 |
| Capital Investment | 2.0-4.0 | 1.20-2.10 | 1.60 |
| Labor | 1.90 | 0.60 | 0.60 |
| Total | 9.2-14.4 | 6.9-11.2 | 6.8 |

## SECTION IX

## SUMMARY

## AMBER LABORATORIES

The experimental data and experience obtained operating the demonstration pilot plant over an extended period of time, leads to the following observations:

Saccharomyces fragilis may be grown on an acid (or sweet) whey medium in a continuous, deep tank, aerated, fermentor. While similar fermentations have been described and demonstrated in the literature for some time, this study has shown that variations in fermentation conditions, strain selection and medium composition produced cell counts of several billion cells per milliliter, that may be maintained for extended periods of time.

The fermentation itself has many advantages easily recognized by the experienced investigator. By operating at a low pH (4.5) and with a large seed size and a high cell count, contamination is no problem and therefore, sterile or special aseptic equipment or techniques are not necessary. The aeration requirements are not excessive with adequate agitation and efficient baffling, nor is there any problem in foam control. Wang (10) reported that hydrocarbon fermentations required 2.5-5.0 times the amount of oxygen as a carbohydrate fermentation. Temperature control, despite the rapid growth rate, was surprisingly easy and a low level of cooling water was needed. The medium is simple in composition and, at the

concentrations tested, the carbohydrate (lactose) is completely soluble. The absence of potential toxic substances in the medium eliminates the necessity of harvesting the cells by centrifugation. Thus the production of a dried whole fermented mass precludes additional processing of waste streams from yeast separators and increases the yield of the fermentation. As a result of the evaporation process of the whole fermented mass prior to drying, condensate water is obtained that can be used to dilute incoming condensed whey and thereby operate a completely closed system with no effluents.

However, if a high protein, food grade yeast is desired, the cells may be harvested from the medium by centrifugation. The yeast cells are sufficiently large for efficient centrifugation on standard yeast separators.

These products may be obtained from the fermentation of whey and are described as follows: A dried whole fermented whey mass (FWM), a dried cream obtained from the centrifugation process of the FWM, and a dried cream from centrifuged, washed, FWM. A dried whole FWM would make a good feed ingredient. It has a crude protein of 40-50%, a light color, a free flowing characteristic and a pleasant "dairy" odor. The protein shows an excellent amino acid profile, high in lysine, although somewhat low in the sulfur containing amino acids. The quality, as indicated by PER determined in rat feeding

tests, is good (70% of casein PER) although it is much better if the fermented whey mass is centrifuged (90% of casein PER).

Actual feeding tests with animals would have to be run to determine its true value as a feed ingredient. Saccharomyces fragilis is an accepted, non-toxic material and may be used in feed formulations.

When the cells are harvested by centrifugation and the creams dried or when the creams are further washed and centrifuged, two superior products are obtained. The dried creams have a lower ash content than the dried FWM and a PER almost equivalent to casein (PER for centrifuged FWM-2.26; PER for casein 2.50). The uses for such a product may be in specialty feeds that can pay for the added cost of processing. The washed and dried cells, give an excellent food grade yeast that compares favorably with the protein and ash levels (47% protein and 7% ash) of Torula and Debittered Brewers Yeasts now being sold commercially.

The economics of the fermentation is dependent on many factors and should compare favorably with other procedures for the production of single cell protein. By beginning with a soluble, inexpensive carbohydrate source (lactose in whey) many distinct advantages should be recognized over those processes that use hydrocarbons as the carbon substrate. A few of the advantages are lactose is more soluble than hydrocarbons; lactose

fermentations require 1/3 to 1/2 the amount of oxygen necessary for hydrocarbon fermentation; lactose fermentation broths do not require separation or solvent removal; lactose fermentations require less agitation and cooling than hydrocarbon fermentation. In addition the ease of processing and its present acceptability as a feed and food ingredient are valuable considerations.

The cost of production of such an ingredient is primarily dependent on the cost of whey and the capital investment. Obviously tremendous amounts of excess whey are available in this country. The figures are well known and do not need to be repeated. Excess whey must be removed as a contaminant or a pollution source from our environment and in one way or another the cheese producer will be required to pay the expense of pollution abatement. For these reasons the cost of whey to a whey processor will be low or non-existent. At the present time, there are a number of cheese producers who pay others (either whey processors or municipalities) to dispose of their excess whey. Therefore, the cost of whey should be realistically set at zero and possibly a negative value in the future.

Another factor that will determine the cost of the final product is the capital investment required. To be truly efficient and competitive, a sophisticated plant is required with a large fermentation capacity. A plant that produces 3,600 tons

of material annually would require an investment of over 5 million dollars. The production of the product in the necessary amounts to be competitive, would require a plant with an annual capacity of 7,000 to 10,000 tons and an investment of 10-15 million dollars.

The economics of the market place will ultimately determine the industrial feasibility of this process. Several factors favor the use of such an ingredient in animal feeds and may be listed as follows: The shortage of high quality protein, the high cost and continued shortage of feed grade non-fat dried milk, and the constant reformulation and special vitamin requirements of the new high energy feeds. The need for such a valuable feed ingredient will continue in the foreseeable future and should increase. The use of the described process should produce material to fill this need as well as provide a useful outlet for a potential pollutant.

## SECTION X

## ACKNOWLEDGMENTS

The support of the sub-contractor, International Minerals Corporation, and in particular Dr. Ralph Anderson, Dr. Martin Rogoff, and Mr. Doug Sisson is acknowledged with sincere thanks.

The following personnel are acknowledged for their valuable assistance in performing the laboratory and pilot plant studies at Amber Laboratories, Mrs. Leslie Oberts, Mr. Oliver Justman, and Mr. Percy Love, who supported the work directed by Dr. Tom Everson and Dr. Sheldon Bernstein.

The support of the project by the Environmental Protection Agency, and in particular Mr. Kenneth Dostal, the Grant Project Officer is acknowledged with sincere thanks.

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## SECTION XII

## GLOSSARY

Acid Whey - The product remaining after removal of casein and fat from cream in the process of making cream cheese, or from skim milk in the process of making cottage cheese.

Amber BYF 100 - Autolyzed yeast fraction: Spray dried yeast supernatant.

Amber BYF 300 - Autolyzed yeast fraction: Spray dried yeast cream.

A.N.R.C. Casein - A standardized casein product adopted for use by Animal Nutrition Research Council for Official Association of Analytical Chemists rat P.E.R. assays.

B.O.D. - Five Day - Biological Oxygen Demand: Procedure performed in accordance with methods for the Examination of Water and Wastewater, 13th Ed., 1971, pp. 489-495.

FD-82 Hodag Antifoam - Fermentation defoamer from Hodag Chemical Corporation, Chicago, Illinois.

Medium 5102A - A whey medium used for production of Saccharomyces fragilis or S. lactis yeast. Medium consisted of whey with a lactose concentration of 6%; 0.5% $(NH_4)_2$ $SO_4$ (w/v) 0.5% $K_2HPO_4$ (w/v) and 0.1% Amber BYF 300.

Medium 5102B - A whey medium used for production of Saccharomyces fragilis or S. lactis yeast. Medium consisted of whey with a lactose concentration of 6%; 0.9% $NH_4OH$ (w/v); 0.3% Amber BYF 300; 0.001% FD-82 Hodag antifoam.

N.U.R.L., Y-1140 - Northern Utilization Research Laboratory, Strain Y-1140, Saccharomyces lactis.

N.U.R.L., Y-1109 - Northern Utilization Research Laboratory, Strain Y-1109, Saccharomyces fragilis.

Protein Efficiency Ratio - Assay Method used to determine efficiency of utilization of nitrogen by rats. Single-cell Protein fed to growing rats as only nitrogen source in an otherwise adequate diet.

Scale Air - Arbitrary method of measuring the amount of compressed air injected into the fermentation vessel.

S.O.P. Medium-1 - A whey medium used for production of Saccharomyces fragilis of S. lactis yeast, medium used for

yeast studies in shake flasks and consisted of whey with a 10% solids content; 0.5% $(NH_4)_2 SO_4$ (w/v); 0.5% $K_2HPO_4$ (w/v); and 0.3% Amber BYF 100 (w/v).

S.O.P. Medium - 2 - A whey medium used for production of Saccharomyces fragilis or S. lactis yeast. Medium used for yeast studies in New Brunswick and 500 gallon fermentors and consisted of whey with a 10% solids content: 0.9% $NH_4OH$ (w/v); 0.3% Amber BYF 100 (w/v), 0.05% $H_3PO_4$ (w/v); and sufficient 30% HCl to adjust pH to 5.5 for S. lactis and to 4.5 for S. fragilis fermentation.

Sweet Whey - The product remaining after removal of casein and fat from milk in the process of making Italian, Swiss and Cheddar Cheese.

## SECTION XIII

## APPENDICES

| | | | |
|---|---|---|---|
| Title: | Whey Fermentation | Investigators: | M. Rogoff<br>N. Janosko<br>D. Sisson |
| Project: | Contract Research -5000 | Location: | Libertyville |
| Cost Center: | Microbiology ) 0741 | Period: | Oct-Dec 1971 |

Objective:

Develop an economic process to convert whey lactose to yeast protein without deproteinizing the whey. A high quality protein feed supplement is the desired product.

Background

Processes based on utilization of whey lactose by yeasts on deproteinized or whole whey substrates have been previously described (1). Some result in rather efficient utilization of whey lactose, e.g. the process described by Wasserman while others are apparently less so. Among such processes in the latter category are the "Wheast" process (2). that of Metwally et al (3) and that of Amundson (4). If Wasserman's 24 g/litre yield of yeast is taken as approaching the theoretically feasible yield of yeast (55% weight conversion from lactose) then the other processes might be ranked Wheast, Metwally and Amundson in decreasing order of efficiency.

The more efficient processes in terms of lactose conversion are characterized by a short cycle time, 6-8 hours, and a single doubling of the yeast cells in the fermentor. This type of process requires a 50% inoculum to the fermentor. Use of such a high inoculum level has several drawbacks in operation of an economical fermentation process as follows:

1) The seed vessel must be at least half the size of the fermentor.

2) Inoculum to the seed vessel must be sufficient to complete seed development in a time s fermentor cycle time. Unless this criterion is met, more than one seed vessel per fermentor or a semicontinuous seed operation is required.

3) The entire seed train must be high volume or long in duration to obtain the required number of cells.

4) If operated as in the "Wheast" process requires a centrifuged seed which constitutes an additional operating cost and is equivalent to recovering half the volume of the fermentor twice.

5) The economics of operating with the fermentation/turnaround time ratio very low may be unfaverable.

The feasibility of operating a process based on a 50% inoculum level is minimally questionable, and not representative of normal fermentation operations. The latter are usually operated at a nominal 15% maximum inoculum level.

The present study was undertaken in order to demonstrate operation of a whey lactose to yeast conversion process which would be commercially feasible and reproducible for ready scaleup.

Status at Beginning of Quarter

Project initiated this quarter.

Progress During Quarter

Shake flask studies were completed for determination of the following:

1. Strain. Six yeast strains were screened for growth on diluted whey concentrate. Two cultures Saccaromyces lactis NRRL Y-1140 (S. lactis H.) and S. fragilis NRRL Y-1109 (S. fragilis W) were retained for scaleup to pilot plant fermentors.

2. Medium. Effect of N-level and Yeast Extract additions were tested. SOP for these to FPP was 0.5 and 0.1%, respectively.

3. Effect of carbohydrate level. Serial feeding of lactose was tested to determine whether sugar was limiting in the system. An indication of increased cell numbers was obtained by pure lactose additions either in the batch or by serial feeding.

4. Growth rate. Generation times were examined under several conditions. It was found that at 10% inoculum levels one log increase could be obtained in 8 hours to yield maximum population obtainable on or 5% lactose in flasks. These data indicated feasibility of an 8 hour fermentation time in larger equipment thus 3 cycles/24 hour period.

## Results and Discussion

**Methods.** Shake flask experiments were carried out as follows:

1) Stock cultures were maintained on whey agar slants (5% lactose) pH 5.0 and transferred at weekly intervals.

2) Inocula were prepared by washing the growth from a 24 hour whey agar slant into 5 ml of whey medium and inoculating 1.5 ml into 50 ml of whey medium in a 250 ml flask. Flasks were incubated on a rotary shaker at 270 rpm at 30°C. Media were not sterilized in any of the procedures used with the exception of whey agar for stock slants.

3) Shake flasks were charged with 90 ml of whey medium prepared as follows: whey concentrate diluted to 5% lactose (or as indicated in individual experiments) and 0.5% $(NH_4)_2SO_4$, 0.5% $K_2HPO_4$ and 0.1% yeast extract added; pH as indicated in individual experiments. Flasks were inoculated with 10 ml of 16 hour growth from suitable inoculum flasks. All incubations were at 270 rpm, 30°C. Optimal pH for T. lactosa was 3.5. S. lactis 5.5 and S. fragilis 4.5. These pH's were used for the respective cultures.

4) Analytical. a) Cell count. microscopically, by standard dilution technique in a blood counting hemocytometer, b) Cell pack - 10 ml of broth were spun down in volumetric tapered centrifuge tubes for 30 minutes at 3000 rpm. Volumes were read directly.

5) Reducing sugar (lactose) values were determined by a standard Technicon Autoanalyzer colorimetric method.

6) Dry weights were determined by shell freeze drying of appropriate aliquots of broth.

## Experimental

The shake flask experiments described below were set up following initial pure culture isolation of strains from incoming cultures and preliminary demonstrations of the ability of raw diluted whey concentrate to support growth. The first five experiments were carried out using all six strains of yeast originally obtained. These included: Torula lactosa NRRL Y-196; T. lactosa NRRL Y-1203; Saccharomyces lactis NRRL Y-1140; S. fragilis NRRL Y-1156; S. fragilis NRRL Y-1109, S. fragilis 1208 (from T. Everson). All subsequent experiments in shake flasks included only T. lactosa Y-196, S. lactis Y-1140 (S. lactis H) and S. fragilis Y-1109 (S. fragilis W).

Shake flask experiments were designed to investigate factors limiting the growth of the yeast. Following preliminary experiemnts to determine optimal pH for each test culture a shake flask standard condition was set. Carbohydrate level was investigated first. Experimental design was to supplement whey concentrate diluted to a carbohydrate level of 5% with pure lactose in five percent increments. Carbohydrate supplementation was made by either a batch procedure (SF 2,3) or by a serial feeding of 5 percent increments at 8 hour intervals (SF 4). An almost direct response to carbohydrate level in terms of cell numbers obtained was observed for the T. lactosa, S. lactis R (Figure 1). Response of S. fragilis to increased carbohydrate was not observed consistently although a response trend might be extrapolated from the data. T. lactosa did not utilize all carbohydrate provided; the other two cultures evidenced usually less than 1% residual carbohydrate in the broth.

The effect of nitrogen level and yeast extract supplementation was investigated in an experiment in which SOP condition was followed and an additional 5% lactose added to the flasks after 8 hours to allow full growth potential (SF-5). Although full utilization of carbohydrate was obtained no differences in cell pack volume attributable to increased N-levels were observed. Since all carbohydrate was utilized it was assumed other factors which might be limiting on the system e.g. dissolved oxygen, might be repressing a nitrogen-response. Pursuance of this line of experimentation was accordingly postponed until operation in pilot plant fermentors was initiated and where $O_2$ availability might be less limiting. Investigation of P effect was also postponed.

A series of shake flask experiments were next carried out to determine whether lactose in excess of 5% provided as additional whey would evoke the same growth response as found on addition of pure lactose (SF 6-7). Experimental format used was dilution of concentrated whey to desired carbohydrate level (5-10%) with no further sugar addition. N and Yeast Extract supplement levels were varied. The results of these experiments indicated that suboptimum growth was obtained and that incremental growth response to carbohydrate was not observed. Providing additional carbohydrate as whey is apparently increasing the amount of some non-carbohydrate material in the medium to an inhibitory level. Under this apparent inhibition no response to adjuncts was observed. S. lactis was perhaps more sensitive to the inhibitory effect than was S. fragilis.

Generation times for the shake flask fermentation were calculated from cell counts taken on flask populations under various conditions. Since it had been observed that carbohydrate depletion had taken place usually within 8 hours

generation times calculated were all based on an 8 hour period. Some of these data are summarized in Table 1. Slightly over 3 doublings are required to bring a 10% inoculum (of maximum cell growth) to original cell levels; in short one log increase in cell numbers. Examination of count data indicated 2.8-3.2 doublings of populations of S. lactis and S. fragilis were obtained in 8 hours. This information indicates that 3 fermentor cycles per day may be feasible.

The data in Table 2 are a general summary of shake flask data obtained. Details of individual experiments are recorded in IMC Laboratory Notebook #5587 assigned to N. Janosko.

## Future Plans

1. Initiate Pilot Plant fermentor studies on shake flask SOP. Characterize population response.

2. Investigate effects of N, Yeast or Yeast Extract, P and carbohydrate levels in fermentors.

3. Investigate effects of physical parameters, e.g. , $O_2$, agitation, on the fermentation.

4. Investigate effects of inouculum levels on yeast yield. Investigate effect of retention of an aliquot of fermentor final whole culture as inoculum on semi-continuous operation of the process.

5. Initiate characterization of yeast or product yields in terms of carbohydrate, protein, fat, ash and moisture under various fermentation conditions. Carry out amino acid analysis on selected representative product samples.

## References


1. Wasserman, A.E., Appl. Microbial. 1960, 8 (5), 291.

2. Robe, K., Food Processing, Chicago, 1964, 25 (2), 95

3. Metwally, M.E., et al., J. Dairy Sci., 1964, 47 (6), 680.

4. Amundson, C.H., Amer. Dairy Rev., 1967, 29 (7), 22.

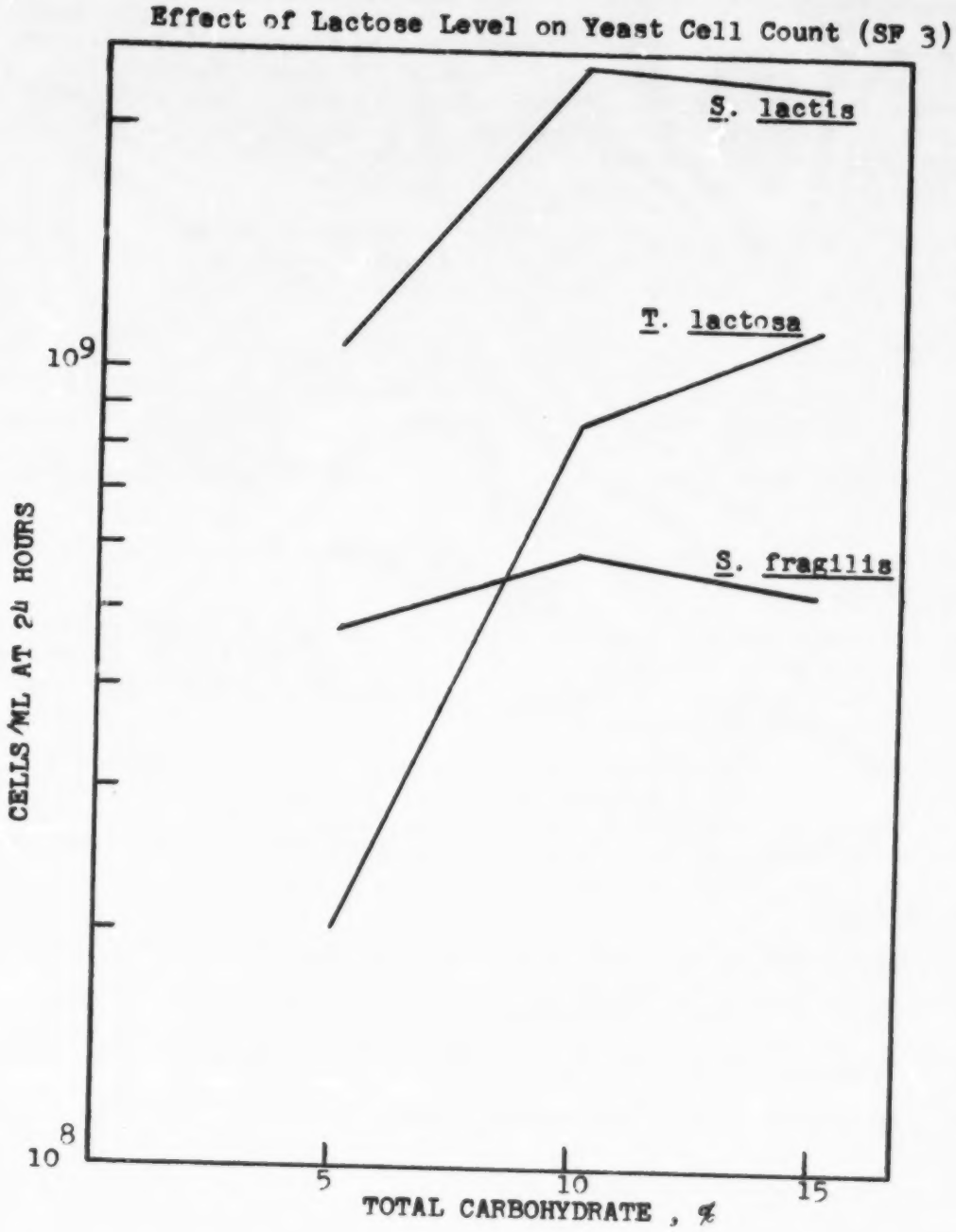

Effect of Lactose Level on Yeast Cell Count (SF 3)
S. lactis
T. lactosa
S. fragilis
$10^9$
$10^8$
CELLS/ML AT 24 HOURS
5
10
15
TOTAL CARBOHYDRATE , %

TABLE 1. Yeast Population Increases During 8 Hour Fermentations Under Various Condtitions

| Condition | Yeast | Initial cells/ml $x10^8$ | Final cells/ml $x10^8$ | No. Doublings |
|---|---|---|---|---|
| SOP (see text) | T. lactosa | 0.27 | 2.04 | 3.0 |
| | S. lactis | 0.96 | 10.75 | 3.4 |
| | S. fragilis | 0.33 | 4.75 | 3.8 |
| SOP | T. lactosa | 0.37 | 2.3 | 2.6 |
| | S. lactis | 1.1 | 17.5 | 4.0 |
| | S. fragilis | 0.27 | 6.7 | 4.5 |
| SOP, Whey adjusted to [lactose] | | | | |
| = 12.6% | T. lactosa | 0.54 | 0.85 | 0.6 |
| = 10.4 | T. lactosa | 0.54 | 1.10 | 1.0 |
| = 7.5 | T. lactosa | 0.54 | 2.0 | 1.9 |
| = 6.0 | T. lactosa | 0.54 | 2.15 | 2.0 |
| = 12.6% | S. lactis | 1.2 | 7.05 | 2.5 |
| = 10.4 | S. lactis | 1.2 | 6.45 | 2.3 |
| = 7.5 | S. lactis | 1.2 | 8.35 | 2.8 |
| = 6.0 | S. lactis | 1.2 | 7.55 | 2.6 |
| = 12.6% | S. fragilis | 0.32 | 3.1 | 3.2 |
| = 10.4 | S. fragilis | 0.32 | 4.05 | 3.6 |
| = 7.5 | S. fragilis | 0.32 | 3.25 | 3.3 |
| = 6.0 | S. fragilis | 0.32 | 4.85 | 3.9 |

AVERAGE GENERATION TIME:

T. lactosa = 3.2 hrs

S. lactis = 2.4 hrs

S. fragilis=2.0 hrs

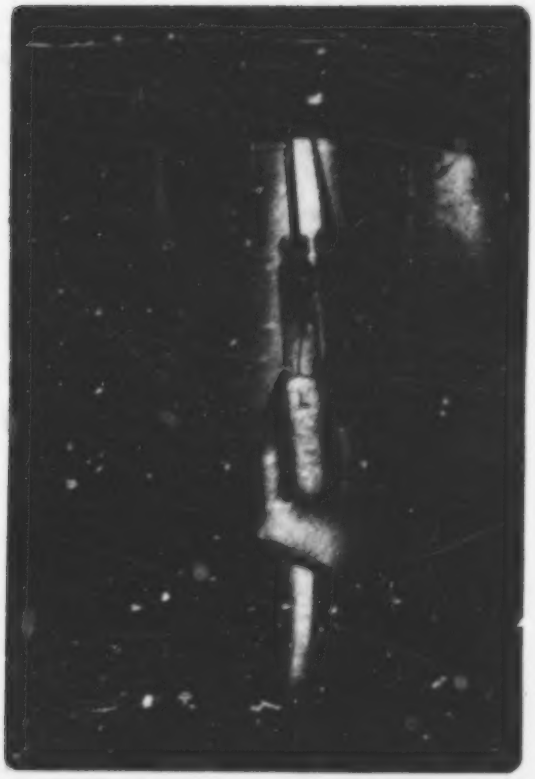
TABLE 2. Summary of Shake Flask Experiments*

| # | Experimental Conditions | T. lactosa Y-196* Cells x10^8 | Resid CHO % | Pack ml T** | S. lactis* Cells x10^8 | Resid CHO % | Pack ml T** | S. fragilis W* Cells x10^8 | Resid CHO | Pack ml T** |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | SOP | 2.9 | | 0.35 | 10.5 | | 0.55 | 4.9 | | 0.5 |
| 2 | SOP ) Batch | 2.0 | | | 10.2 | | | 5.2 | | |
| | SOP + 5% lactose ) Batch | 1.7 | | | 7.6 | | | 3.6 | | |
| 3 | SOP | 2.04 | 3.76 | | 10.8 | .37 | | 4.8 | .29 | |
| | SOP + 5% lactose ) Batch | 8.5 | .54 | | 24.0 | .40 | | 5.9 | .44 | |
| | SOP + 10% lactose ) Batch | 11.5 | 4.20 | | 22.5 | .44 | | 5.3 | 3.23 | |
| 4 | SOP ) | 2.3 | 3.7 | .30 | 17.5 | 1.02 | .36 | 4.7 | .66 | .50 |
| | SOP + 5% lactose ) 5% serial feed | 10.95 | 4.8 | .62 | 24.7 | .47 | .64 | 8.5 | .36 | .70 |
| | SOP + 10% lactose ) | 18.6 | 4.3 | .77 | 20.1 | .34 | .64 | 6.8 | 1.58 | .60 |
| 5 | SOP - As Is H4 Whey ) | | 0.60 | .75 | | 0.56 | 1.025 | | 0.39 | .775 |
| | SOP c 2x $(NH_4)_2SO_4$ ) As Is | | 0.49 | .775 | | 0.40 | 1.15 | | 0.36 | .85 |
| | SOP c 2x Yeast Extract) | | 0.54 | .80 | | 0.42 | 1.1 | | 0.51 | .70 |
| 6 | SOP = Conc. whey adjusted to 10% CHO | | | | | | | | | |
| | SOP + 0.4% N .05% Y | | | | 5.6 | 8.1 | 0.7 | 2.7 | 8.3 | 0.6 |
| | SOP + 0.5% N 0.1% Y | | | | | 8.9 | .675 | | 9.6 | 0.5 |
| | SOP + 0.6% N 0.15% Y | | | | | 9.6 | 0.75 | | 9.25 | 0.5 |

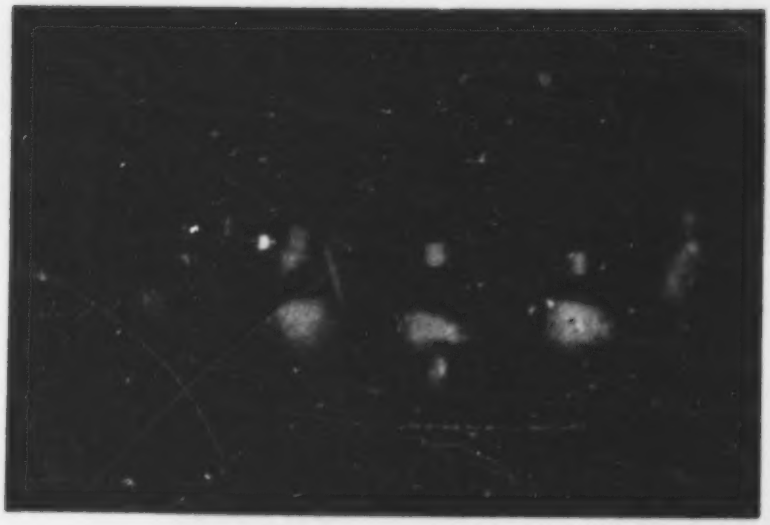
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | Dilution of whey to various CHO levels | | | | | | | | | |
| | CHO | 10.0 | N 0.5 | Y 0.1 | 7.05 | 6.1 | .65 | 3.1 | 6.1 | .6 |
| | | 10 | - | - | | 5.8 | .65 | 3.4 | 5.9 | .68 |
| | | 7.5 | 0.5 | 0.1 | 6.45 | 3.1 | .65 | 4.1 | 2.4 | .6 |
| | | 7.5 | - | - | | 2.1 | .60 | 2.8 | 2.2 | .6 |
| | | 6.0 | 0.5 | 0.1 | 8.75 | .46 | .625 | 3.3 | - | .5 |
| | | 6.0 | - | - | | .48 | .60 | 3.6 | - | .5 |
| | | 5.0 | 0.5 | 0.1 | 7.55 | - | .60 | 4.9 | - | .55 |
| | | 5.0 | - | - | | .05 | .60 | 5.8 | - | .55 |

* Minimally duplicate standard triplicate flasks were set for most experimental conditions. Data obtained with three other yeast cultures in the first five experiments are not included above (see text).

** ml wet cells/10 ml broth

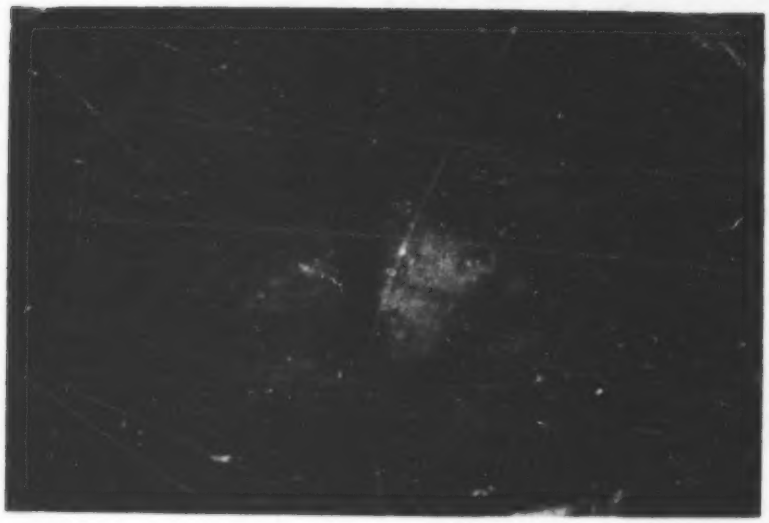


Live culture of yeast asceptically transferred from yeast agar slant to shake flask.



Growth of yeast on whey media in shake flasks.

Growth of yeast on whey media in New Brunswick fermentors.

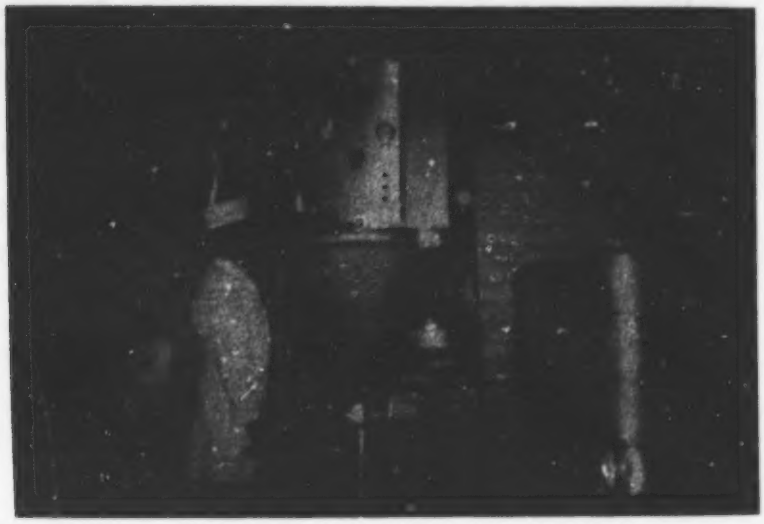


Top view of 500 gallon fermentor showing mechanical foam breaker and yeast/whey fermentation broth.

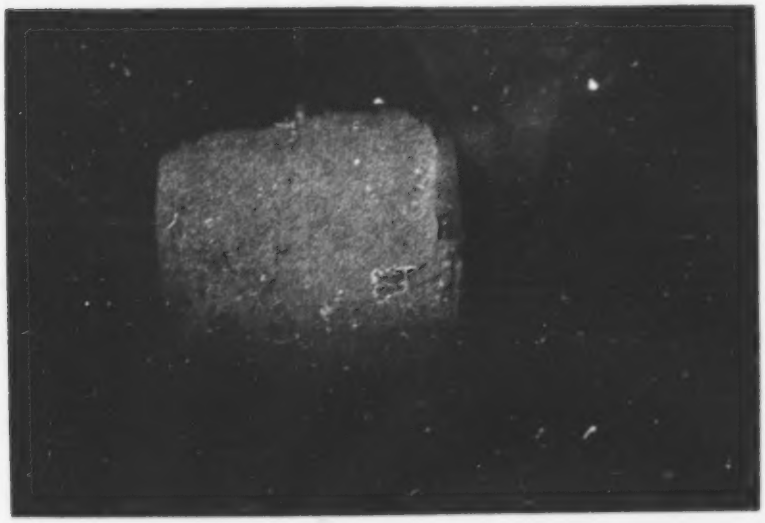
Broth from continuous yeast fermentation of whey.

Growth of yeast on whey media in 500 gallon deep tank fermentor.

Progress of yeast fermentation followed by actual cell counts.



Pilot spray dryer for production of experimental lots of dried yeast.



Spray dried yeast from continuous growth of yeast on whey.